# FIELD APPLICABLE METHOD TO REDUCE DENTAL EMERGENCIES
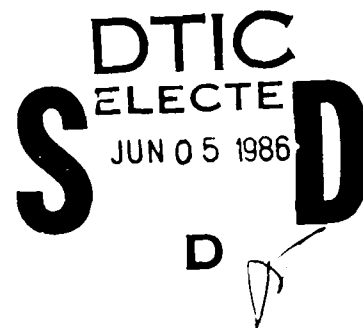
Annual Report

By

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In vitro experiments have shown that twice daily exposure of S. mutans to various fluoride salts has shown that only $SnF_2$ significantly alters this organisms growth and metabolism. The antibacterial effect of $SnF_2$ was associated with an uptake of tin into the bacteria. Fluoride salts of sodium, lead, zinc, and copper had little effect in this test system. The pH of the various fluoride salts generally had no effect on the test compound activity except for the noted inactivation of $SnF_2$ at elevated pH's. Since $SnF_4$ also did not affect the growth or metabolism of S. mutans, a unique property of $SnF_2$, possibly its reactivity in an aqueous environment, may be responsible for its antibacterial properties.

Stannous fluoride was compared to NaF (5 ppm $F^-$) in the drinking water of hamsters to test whether $SnF_2$ had greater caries inhibitory effects due to its potential antibacterial effects. The number of enamel and dentinal carious lesions in both the NaF and $SnF_2$ group was significantly different from the deionized water group; however, there was no difference in caries scores between the NaF and $SnF_2$ group. The recovery of S. mutans was highly variable between animals and showed no statistical difference between groups.

Twenty-two human subjects, who were regarded as potentially caries active, rinsed twice a day with either acidulated NaF or $SnF_2$ mouthrinses, adjusted to 200 ppm $F^-$. There was a small (2 times) but significant reduction in Total CFU per ml saliva in both groups after a year. No differences were found in lactobacilli counts between the 2 mouthrinse groups or longitudinally within the groups. Of importance the apparent selective reduction in S. mutans found in those subjects rinsing with $SnF_2$. At the end of 1 year, the $SnF_2$ group had less (26 times) fewer S. mutans compared to baseline. With regard to caries, all patients continued to be caries active after one year despite the use of two daily fluoride mouthrinses; however, the subjects rinsing with $SnF_2$ developed approximately half the number of new carious lesions to those subjects rinsing with acidulated NaF. With regard to gingival health, this study did find that $SnF_2$ was an adjunct in decreasing gingival inflammation. The lower frequency of bleeding sites and the corresponding lower mean GI scores in the $SnF_2$ group compared to the NaF group demonstrates that rinsing with $SnF_2$ favorably affected gingival health.

The controlled release clinical trials were designed to examine the safety and efficacy of a controlled release delivery system of $SnF_2$ in a small number of human subjects. The $SnF_2$ restorations showed no signs of wear or loss of integrity in both the trials. The salivary fluoride release rate was found to average 0.3 ppm over the 34 day trial. Some effect on both the quantity and proportion of microorganisms was noted in those subjects who had a $SnF_2$-polycarboxylate restorations in place. While there was an increase of recovery of total colony forming units from salivary in the placebo group during the experimental period, probably due to suspension of oral hygiene in this period, a decrease in total bacteria was noted in the $SnF_2$ group. This decrease in salivary microorganisms may be selective since S. sanguis recoveries showed no difference between groups while S. mutans recoveries appeared less in those subjects having the $SnF_2$ restoration. The effect of the $SnF_2$ delivery system against plaque and gingivitis was not impressive.

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# Summary

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STUDY 1: DETERMINANTS OF THE ANTIBACTERIAL EFFECTS OF $SNF_2$

AGAINST S. MUTANS: IONS, pH VALENCE

## Introduction

Fluoride compounds have been used topically in the oral cavity for many years with the intention of affecting tooth enamel to alter its resistance to dental caries. Fluoride may also affect the bacteria in the mouth. Bibby and van Kesteren (1942) found that 1 ppm $F^-$ as NaF reduces bacterial acid production. While there are some antibacterial properties of NaF, $SnF_2$ has been shown to have significantly more affect against oral microorganisms in vivo and in vitro. Recently $SnF_2$ has been shown to selectively reduce S. mutans, the bacterium associated with dental caries.

The difference between NaF and $SnF_2$ in affecting oral bacteria have been suggested to be due to: 1.the divalent cation ,Sn, competing with calcium to alter bacterial adhesion/cohesion; 2. tin oxidizing the thiol groups of bacterial enzymes; 3. the large uptake of tin disrupting bacterial metabolism; or 4. the naturally low pH of $SnF_2$ which would allow HF formation and thus be more antibacterial.

The purpose of this report was to compare $SnF_2$ to other compound having similiar ions, pH, valence, or atomic weights to determine what characteristic of $SnF_2$ is necessary to produce the apparent antibacterial affect against S. mutans.

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## Materials & Methods

### Microorganism and Medium

A streptomycin-resistant mutant of <u>Streptococcus</u> <u>mutans</u> NCTC 10449, known to adhere to smooth surfaces <u>in</u> <u>vitro</u>, and to produce dental caries in rats was used in this study. Stock cultures were maintained by monthly transfer in fluid thioglycollate medium (Difco) supplemented with 20% v/v meat extract and excess $CaCo_3$. For experiments, cultures were adapted and grown in the complex medium of Jordan et al. (1960), supplemented with 50 mg of $Na_2CO_3/l$ and containing 5% sucrose (pH 7.5).

### Experimental Design

Stainless steel wires (0.5 mm diameter), suspended in culture tubes by rubber stoppers, were used as a substratum for the bacterial plaques. For plaque growth, 10 ml of the complex medium was inoculated with 0.1 ml of the adapted S. mutans cultures and the wires were initially colonized by inoculating them in the medium for 12 hr. at $37^O$. Intermittent exposure to the various test agents (Table I), starting after 12 hr. growth, involved removing each wire from the medium, placing them into the appropriate test solution for 1 min., reducing carryover of test solution with a 1 min. non-agitated rinse in 10ml $H_2O$ (pH 6.0), and then placing the wire into 10 ml of fresh growth medium. This exposure of the adherent plaques to the test solutions was repeated 2 more times at 12 hr. intervals.

All experiments were terminated after 48 hrs., 12 hrs. after the last exposure to the test agents. The thickness of the adherent plaques was visually scored by comparing the growth to standards. Except for those samples processed for electron microscopy, the plaques from each wire were

collected into pre-weighed tubes, pelleted by centrifugation, dried for 3 days at 70°, and weighed. The pH of the growth medium representing 12 hr. growth was also determined at the end of the experiments.

An exception to the above procedure took place in one trial the purpose of which was to test the effect of agents on "pre-formed" plaque. In this experiment, the only difference was that plaque was allowed to form on the wires for 48 hours before they were exposed to the test agents. In this case, the experiment was terminated on the 4th day.

## Atomic Absorption Spectrophotometry

Dried samples were further processed to determine their metal content. Tin quantity was determined by an atomic absorption using a spectrophotometer (Perkin-Elmer, Model 403) equipped with a graphite furnace (Model HGA-74). Lead was also determined with the aid of the graphite furnace. Zinc and copper was quantified by the method of addition using flame atomic absorption spectrophotometry. A deuterium lamp was used in all cases to correct for non-atomic absorption signals.

## Transmission Electron Microscopy

Bacterial specimens designated for electron microscopy were fixed on their wires at the end of the experiment (48 hr) with 2.5% gluteraldehyde in phosphate buffer (pH 7.4, 390 mOsm) and postfixed in 1% osmium tetroxide in veronal buffer (pH 7.3). The fixed bacteria were then removed from the wires, washed in phosphate buffer, dehydrated in acetone and embedded in epoxy medium. Thin sections were prepared with a LKB ultramicrotome using a diamond knife. Silver-gold colored sections were examined unstained with a Zeiss EM10 electron microscope at 80 kV.

Energy-dispersive x-ray analyses, to confirm the presence of specific

metal deposits In or on the bacteria, were performed in a JOEL JEM-100 CX transmission electron microscope equipped with a high resolution electron microscope accessory (ASID) and a Kevex SI (LI) x-ray detector connected to a Micro-X Analytical X-ray Spectrophotometer, Model 7000.

Results

An initial trial (Table 2), comparing the intermittent exposure of four fluoride containing solutions at their natural pH's showed that $SnF_2$ had several effects on the growth of adherent S. mutans cultures compared to the other fluoride solutions. Obvious differences in plaque scores, dry plaque weights, and acid production were found on all the wires exposed to $SnF_2$. No differential growth was noted in those plaques exposed to NaF, $ZnF_2$ or $SnF_4$. The metal content of the dried plaques was also variable among the treatment groups. There was little metal content in those samples exposed to $ZnF_2$. Those samples exposed to $SnF_4$ had tin present, but not nearly as great as those plaques exposed to $SnF_2$. In the $SnF_2$ samples, approximately 4% of the plaque dry weight could be attributed to tin.

Another trial was performed in an attempt to discriminate whether the pH, the fluoride, or the tin content could account for the noted growth inhibition of the $SnF_2$ solution (Table 3). In this trial, even though $SnF_2$ at pH 2.5 had antibacterial effects, there was no growth alteration apparent due to fluoride, pH or due to tin, per se. The pH of the $SnF_2$ solution was critical. Since the $SnF_2$ solution adjusted to pH 7.0 had no effect.

To further understand the effect that the pH of $SnF_2$ had on the observed antiplaque properties, $SnF_2$ was prepared with in a range of pH's from 2-6, and tested in the same bacterial growth system (Table 4). $SnF_2$ appeared to have more growth inhibition at lower pH's, with those plaques exposed to $SnF_2$ below pH 4 being most affected. Besides the low pH of $SnF_2$ solutions producing the most growth inhibition, plaques exposed to these solutions had the most metal content per mg plaque (Table 4, Figure 1).

Two other metallic fluoride compounds were also compared to NaF to examine whether other compounds would have similar effects to $SnF_2$. Neither $PbF_2$ or $CuF_2$ at the 2 pH's examined showed any effect more than NaF at comparable pH levels (Tables 5 & 6).

The possibility was also explored that established bacterial colonies would affect the antibacterial properties of $SnF_2$. S. mutans plaques were allowed to preform on the wires for 2 days before being exposed to $SnF_2$. While there was some reduction in visual plaque score and acid production in those samples exposed to $SnF_2$, no difference in plaque weight was noted after the weight of tin was subtracted from the plaque dry weight (Table 7).

Electron micrographs of those bacterial specimens exposed to $H_2O$, NaF, $SnCl_2$, $ZnCl_2$ and all appeared similar with morphologically normal gram-positive cocci surrounded by extracellular material. However, the bacteria exposed to $SnF_2$ had frequent electron-dense granules, most often within the bacterial cell, but occasionally on the outer cell wall. Energy-dispersive x-ray microanalysis of this electron dense material revealed peaks corresponding to the $L\alpha$ (3.67 KeV) and $L\beta$ (3.44 KeV) peaks for tin. Besides the electron dense areas, the bacteria exposed to $SnF_2$ frequently were also noted to have intracellular electron-lucent holes and distorted all shapes (Figure 3). A unique feature of the specimens exposed to lead fluoride was the presence of electron dense granules located only on the outer cell wall (Figure 4). Electron microprobe of these granules confirmed them to be lead ($L$ = 10.50, $L$ = 12.62 KeV).

## Discussion

The results in these series of experiments confirm our earlier findings that $SnF_2$ has antibacterial properties against S. mutans, and this effect appears related to the uptake and retention of tin by this organism. However, the present studies show that compounds similar to $SnF_2$ in pH, ions, or valence have little or no effect on S. mutans growth or colonization.

While others have reported that an acidic solution of NaF has some antibacterial properties due to the cellular uptake of hydrogen fluoride, our in vitro model using intermittent exposures to test agents showed only slight reduction of acid production and no growth inhibition in those bacteria exposed to NaF at a low pH. The only apparent effect that pH alteration had on the test solutions was that noted with $SnF_2$. In all growth parameters tested, there was an inverse relationship of the pH to $SnF_2$ solutions and its effectiveness as an antibacterial agent. $SnF_2$ solutions near neutrality showed no antimicrobial properties. Hydrolysis of $SnF_2$ solutions at elevated pH's probably results in the reduced antibacterial properties.

The degree of bacterial colonization of the wires at the time of the initial $SnF_2$ exposure was also an important parameter in the effectiveness of $SnF_2$. In our experiments, we allowed 12 hours for the wires to become colonized by S. mutans before the first exposure to the test solutions. Bacteria that had less time to colonize the wire had essentially no growth after the exposure to $SnF_2$. Conversely, heavy bacterial colonization of the wires, as with preformed plaque, reduced the effectiviness of the 1 minute exposures to $SnF_2$. Preformed plaques would not visually grow, but they still were metabolically active as shown by their ability to reduce

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the pH of the growth medium. The decreased effectiveness of $SnF_2$ with preformed plaques agrees with previous findings in vitro and in vivo. Perhaps a thick bacterial mass reduces diffusion of this antibacterial agent.

Unexpected in this study was the finding that only $SnF_2$ showed antibacterial properties against S. mutans. Metal salts, especially those with high atomic weights, are generally regarded to have antibacterial properties. $SnCl_2$, however, has been previously noted to have little antimicrobial activity. This compound is unstable in aqueous solutions which probably reduces the available tin to the bacteria. The fluoride salts of lead and zinc were tested because they, like $SnF_2$, are divalent cations, with $PbF_2$ having a greater atomic weight and atomic diameter than $SnF_2$, and $ZnF_2$ being lighter and smaller. Although plaques exposed to $PbF_2$ in this study had quantative metal uptake, the electron micrographs showed the lead to be located only on the outer cell wall. No alteration in bacterial growth parameter due to $PbF_2$ was observed. Other reports have also found that bacterial cell membranes can bind lead with no detectable effect on growth. $ZnF_2$ also showed no effect on bacterial growth, but in contrast to $PbF_2$, no zinc binding in or on the bacteria was noted. Other investigations have shown little inhibition of bacterial growth by zinc compounds.

Surprisingly, no effect on bacterial growth was noted for $CuF_2$ or $SnF_4$. $CuF_2$ has been reported to be effective in reducing bacteria acid production in vivo. In the present study, there was no measurable reduction in bacterial acid production by $CuF_2$, nor did $CuF_2$ alter any other bacterial growth parameters. $SnF_4$, although showing no effect on the growth of S. mutans, produced some quantative retention tin in the cells. The electron

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micrographs, however, showed that with SnF$_4$, the electron dense deposits were located only on the surface of the cells.

The apparent unique antibacterial properties of SnF$_2$ thus appears to be assiciated with the observed intracellular retention of tin. The high percentage of tin in the bacteria as measured by atomic absorption spectrophotometry and the large numbers of intracellular tin deposits found in those bacteria exposed to SnF$_2$ suggests that S. mutans cells in some way transports this metal into the cell where it becomes firmly bound. The apparent condensation of the tin into intracellular granules may be an attempt by the bacteria to reduce the harmful effects of this foreign ion. Metalic granules have also been found in eucaryotic cells exposed to metal compounds.

The intracellular tin accumulation, even though in granules, still appear to disrupt S. mutans metabolism as demonstrated by the reduced growth, acid production, and the electron microscopic presence of holes in many of the bacterial cells. The intracellular holes found in the SnF$_2$ treated specimens may be a sign of unbalanced bacterial growth.

The unique antibacterial properties of SnF$_2$ against this test organism is still not fully understood. No single property of SnF$_2$ which could be isolated (i.e., pH, valence, cations, or size of the molecule) was identified as an important parameter necessary for its effectiveness. SnF$_2$ in aqueous solution is highly reactive and the chemistry of the reactions are not completely understood. It might be that this reactivity or one of the species formed in solution is the important variable which allows for the uptake of tin into the S. mutans cell and the consequent antimicrobial properties.

## CONCLUSION

Twice daily exposure of adherenct $\underline{S.}$ $\underline{mutans}$ to various fluoride salts has shown that only $Snf_2$ significantly alters thais organisms growth and metabolism. The antibacterial affect of $SnF_2$ was associated with an uptake of tin into the bacteria. Fluoride salts of sodium, lead, zinc, and copper had little effect in this test system. The pH of the various fluoride salts generally had no effect on the test compound activity except for the noted inactivation of $SnF_2$ at elevated pH's.

Since $SnF_4$ also did not effect the growth or metabolism of $\underline{S.}$ $\underline{mutans}$, a unique property of $SnF_2$, possibly it reactivity in an aqueous environment, may be responsible for it antibacterial properties.

# STUDY 2:  EFFECT OF NAF AND SNF$_2$ IN DRINKING WATER
## ON HAMSTER DENTAL CARIES

## INTRODUCTION

Besides the unquestionable dental caries reduction produced by fluoride ions, caries has also been shown to be inhibited in man and experimental animals by antiplaque agents.  Several studies have demonstrated that, in addition to its well established physicochemical interactions with enamel, stannous fluoride may also possess antiplaque properties. A recent study has shown that the antimicrobial properties of SnF$_2$ can be demonstrated _in vitro_ even as as low as at 10 ppm F$^-$.

There has been only one study in humans or experimental animals that has compared the caries reduction of SnF$_2$ to NaF.  In that study, fluoride supplementation (18 ppm F$^-$) in water as SnF$_2$ produced a 78% caries reduction in rats, while NaF produced a 52% reduction.  The present study was performed to reexamine whether SnF$_2$ produced greater caries reduction than NaF.  Furthermore, our study was to examine whether the potential antibacterial effects of SnF$_2$ was correlated to a possible greater caries reduction of this compound in an experimental rodent caries study.

METHODS AND MATERIALS

## Animals, Diet, Infection

This study was conducted on 45 "conventional" golden outbred Syrian hamsters. To insure that the effect of NaF and $SnF_2$ was essentially topical, the experiment was begun when the animals were approximately 38 days old. The Syrian hamsters third molars begin eruption on day 30 and occlusion is obtained between the 40-45 day.

On day 1 of the experiment the hamsters were orally inoculated with 0.2ml of a log culture of streptomycin resistant Streptococcus mutans NCTC 10449 in fluid thioglycollate medium; and given the NIH 2000 cariogenic diet and deionized water ad libitum. On day 2, the hamsters were reinoculated, and randomly distributed into deionized $H_2O$, NaF, and $SnF_2$ groups. Fifteen hamsters (5 per cage) received 5 ppm fluoride as NaF (0.0110 g/l, pH 6.5); another 15 received 5 ppm fluoride as $SnF_2$ (0.0207 g/l, pH 4.0); and the third group received water (pH 7.2). The fluoride solutions were made and distributed into plastic feeding bottles each day.

The diet and water continued to be supplied ad libitum until the animals were sacrificed on day 64. The animals were weighed at the beginning and at the end of the experimental period.

## Recovery of Microorganisms

The left maxillary and mandibular molar teeth were used for microbiologic recovery of the streptomycin-resistant infectant. Consecutively, the hamsters were overdosed with pentobarbital, decapitated, and the cheeks and mandibular condyles were cut to enable better access to the jaws. The molar crowns, along with some supporting bone, were excised as a unit with a dental Rongeur and placed into 3 ml of cold 0.05% yeast

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extract broth (pH 7.0). The organisms were dislodged from the teeth by sonication (Bronson Model W185D, Plainville, NY) using a microtip for 40 sec at 50 watts and an output setting of 4.

Serial dilutions were performed using the micro-method of Westergren and Krasse and plated on Mitis Salivarius agar supplemented with 0.001% potassium tellurite and 200 g/ml of streptomycin. After incubation in candle jars for 48 hr at 35°, the streptomycin-resistant S. mutans were counted.

## Caries Scoring

To assess caries, the right mandible and maxilla were defleshed by dermestid beetles. The numbers and extent of the fissure and smooth surface lesions were observed under a dissecting microscope and scored by the method of Konig, modified so that only enamel and dentinal carious lesions were differentiated. Enamel caries was defined as areas of the smooth surface or fissures which had opaque or chalky white areas in the enamel. Dentinal lesions were defined as obvious breaks in the enamel. Proximal surfaces were observed by slightly separating the teeth rather than slicing the teeth mesial-distally which could completely remove the lesions. The jaws were numerically coded so that the experimental histories were unknown to the scorer.

## Statistical Analyses

Differences among group caries scores, microbiological recoveries, and animal weights were tested by analysis of variance using the Scheffe multiple comparison procedure. All tests were performed at the 0.05 level of significance.

RESULTS

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All animals survived and appeared in good health at the end of the experiment. The mean weights and standard deviation per group in grams were: $158 \pm 11$, $148 \pm 14$, and $142 \pm 9$ for the $H_2O$, NaF, and $SnF_2$ groups, respectively. These weights were significantly different suggesting that the fluoride compounds may have had an effect on the weight gains during the experimental period. In 5 animals, minimal microbial recovery of S. mutans from the molars ($<10^4$) resulted in elimination of these animals further analysis.

Table 8 shows the microbial recoveries of the S. mutans at the end of the experiment. The recovery of strain 10449 was variable between animals and neither the actual mean nor the logarithmic transformation of the recovered numbers showed statistical differences between groups.

Table 8 also summarizes the caries data scores and Figure 5 illustrates the scores of enamel and dentinal caries found in the three groups of animals studied. The caries produced in the experiment were mainly small and uncoalesced enabling accurate scoring of the number of lesions. The majority of the carious lesions were of the pit and fissure type with only 11 smooth surface lesions being found. Ten of these smooth surface lesions were found in those hamsters in the deionized water group and one smooth surface lesion in the $SnF_2$ group. The reduction of enamel and dentinal caries in both the NaF and $SnF_2$ groups was significantly different from the deionized $H_2O$ group. The two fluoride groups, however, were not statistically different in any caries parameters examined.

## DISCUSSION

Certain characteristics of experimental caries in hamsters should be recognized when interpreting these results. Dental caries on the smooth

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surfaces of rodents, like man, is dependent on the formation of adherent plaque. However, rodent sulcal lesions are not associated with plaque formation, and may be modified by the impaction of food and debris. Thus an agent used to reduce caries rate by altering microbial growth or adherance, as in the present study, would most likely have the greatest effect on the number of smooth surface lesions where it could exert antibacterial activity.

An example of an antiplaque agent being more effective on smooth surfaces caries is chlorhexidine gluconate, which in a rodent study has been shown to inhibit smooth surface but not sulcal caries. In the present study, essentially only those animals in the deionized water group had smooth surface caries. Therefore, it was not possible to differentiate a potentially greater caries reduction by $SnF_2$, due to its antibacterial properties, from the "fluoride only" effect of NaF on the smooth surfaces.

As expected, the present study also demonstrated that both NaF and $SnF_2$ reduced sulcal carious lesions. Several studies have shown that sulcal caries in hamsters and rats can be significantly reduced by the continuous administration of low levels of fluoride in the drinking water , or higher concentrations applied topically. Our results did not find $SnF_2$ to have a statistically greater effect than NaF on sulcal lesions. Besides the poor potential of antiplaque agents on rodent sulcal lesions, the concentration of the $SnF_2$ was possibly too low to have an antimicrobial effect. Antiplaque properties of $SnF_2$ at mouthrinse concentrations (100-1,000 ppm $F^-$) are known to improve as the concentration of $SnF_2$ increases. Furthermore, very dilute solutions of $SnF_2$, as in the present experiment, may quickly lose soluble stannous ions. Low concentrations of chlorhexidine have also been found to be ineffective in reducing caries in rats. A low

concentration of $SnF_2$ (0.002%) was examined in the present because higher concentrations of fluoride ions (10 ppm $F^-$ as NaF) have been shown to almost entirely eliminate rat caries which would further hinder the possibility of observing differences between $SnF_2$ and NaF.

Another characteristic of experimental rodent caries is the large variability associated with microbial recovery of the infectant. Our technique of excising the crowns _in toto_ and recording the absolute bacterial recoveries rather than relative recoveries, was thought to potentially decrease experimental variance. Yet as with other studies, our data shows large differences in microbial recoveries even among animals in the same group. Possibly the variance is the result of true differences in infection among the animals rather than experimental error.

## CONCLUSION

Stannous fluoride was compared to NaF (5 ppm $F^-$) in the drinking water of hamsters to test whether $SnF_2$ had greater caries inhibitory effects due to its potential antibacterial effects. Caries was produced in the hamsters by orally inoculating them with streptomycin-resistant _S. mutans_, and feeding them NIH diet 2000. After sacrifice on day 64, the hamster's left molars were used for microbial recovery and the right molars were used to assess caries.

The number of enamel and dentinal carious lesions in both the NaF and $SnF_2$ group was significantly different from the deionized water group; however, there was no difference in caries scores between the NaF and $SnF_2$ group. The recovery of _S. mutans_ was highly variable between animals and showed no statistical difference between groups.

The present study demonstrated that both NaF and $SnF_2$ reduced sulcal and smooth surface lesions in the hamster, but the potential difference

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between the fluoride compounds was not evident. It is possible that differences between NaF and $SnF_2$ cannot be shown in a rodent caries model due to: (1) the variability of infection and caries attack among animals; (2) the strong effect of fluoride ion on the caries rates; and (3) the necessary low concentration of the agents tested which may mitigate potential antiplaque effects.

STUDY 3: MICROBIOLOGIC EFFECTS OF $SNF_2$ AND NAF MOUTHRINSES IN SUBJECTS

WITH HIGH CARIES ACTIVITY:  RESULTS AFTER ONE YEAR

## Introduction

The major efforts in the prevention of dental caries have been directed to treatment strategies that affect tooth enamel or to the development of agents that alter the cariogenic microflora. Fluorides, in various concentrations and regimens, have been the most successful agent to date in preventing this disease.  Although the fluoride effect has traditionally been considered to be the result of its physicochemical interaction with enamel, there is evidence that fluoride also alters bacterial metabolism at low concentrations, and is bactericidal at higher concentrations.  Chemical agents that solely affect  bacteria (antibiotics and antiseptics) have also been shown to alter caries activity.  Patients on prophylactic penicillin treatment for medical reasons have been noted to have reduced caries activity.  Chlorhexidine, a potent antiseptic, has also been shown to reduce caries in children.  Attempts to improve caries reduction by combining fluoride with antimicrobials have been only partially successful.  Chlorhexidine diacetate (1%) combined with NaF (0.15%) has been shown to have an additive effect in reducing rat fissure caries, but the combination was not superior to each alone with regard to smooth surface caries.

It has recently become evident that a specific fluoride compound, stannous fluoride ($SnF_2$), has an antimicrobial effect at concentrations compatible with daily fluorice use.  Short term clinical studies with $SnF_2$

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have suggested possible important antimicrobial properties. There is presently no evidence to suggest that these antimicrobial properties would affect Streptococcus mutans and lactobacilli--bacteria correlated to the initiation and to the progression of dental caries in humans.

The aim of the present study was to compare the effects of mouthrinsing with NaF and $SnF_2$ on the number of total aerobic salivary bacteria, S. mutans and lactobacilli numbers in a group of subjects screened for potential high caries activity.

## MATERIALS AND METHODS

### Subjects

The subjects in this study were adults over the age of 18 having incipient carious lesions, high numbers of unrestored carious lesions, and elevated numbers of salivary S. mutans. The 58 subjects were identified by their caries prevalence in the screening clinic at the University of Connecticut School of Dental Medicine. A follow-up microbial screening showed that 38 subjects had greater than $2.0 \times 10^5$ S. mutans per ml saliva. These subjects were regarded as potentially high in caries activity. The 37 subjects who consented to the study were ranked by their recoverable number of salivary S. mutans and then alternately assigned to a $SnF_2$ or an acidulated NaF mouthrinse group. During the first year, 15 patients withdrew from the study. Of the remaining 22 patients, 9 were considered partially compliant with the mouthrinsing procedures. Partially compliant subjects were those who, by their own report, missed more than 4 mouthrinses per month or who were inconsistent with mouthrinsing. We verified these reports by monitoring each patient's remaining supply of

mouthrinse and by questioning the patients monthly for recall of their fluoride usage.

## Treatment

After baseline data were obtained, subjects were instructed to use 10 ml of their respective mouthrinses twice daily for 1 minute per rinse. The $SnF_2$ rinse was diluted with water (1 part rinse; 4 parts $H_2O$) immediately before use to produce a final fluoride concentration of 200 ppm $F^-$ and a pH of 3.4. The acidulated NaF mouthrinse was used full strength at a fluoride concentration of 200 ppm $F^-$ and a pH of 4.0. One month after the initiation of mouthrinsing, each subject received 3 dental hygiene appointments at one week intervals. The oral hygiene instruction, cleaning, scaling and root planing were performed by one dental hygienist, blind to the subjects' treatment categories. The subjects were also assigned to a dental resident for restoration of teeth with active carious lesions. They were contacted monthly to reinforce oral hygiene, to monitor their fluoride usage and to resupply them with mouthrinse.

## Microbiology

Stimulated saliva, produced by chewing on a piece of paraffin wax, was collected from each subject at the screening, at the baseline examination, and after 1, 3, 6 and 12 months. Each saliva sample was sonicated for 1 min., vortexed for 30 sec., and serially diluted from $10^{-1}$ to $10^{-6}$, in 0.05 M phosphate buffer (pH 7.0). From each dilution, 25 $\mu$l was spotted in duplicate on one-third of the surface of an agar plate. The dilutions having 20-100 colony forming units were counted with the aid of 20X magnification. The mean of the 2 samples from these dilutions was used in the analysis.

For cultivation of all aerobic bacteria, (Total CFU) dilutions were spotted or 10% sheep blood agar plates, incubated for 24 hours in a $CO_2-$

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enriched environment (candle jar) at 35°C and counted. For determination of the number of S. mutans, a selective medium consisting of Mitis-Salivarius agar containing 0.2 units/ml Bacitracin was used. After spotting, the agar plates were incubated for 48 hours in a candle jar. Those colonies with morphologic characteristics of S. mutans were counted and their identities confirmed when necessary with biochemical tests. Lactobacilli were cultivated with the aid of Rogosa SL agar plates. The spots were allowed to dry and then a further portion of the agar was poured over the surface. Lactobacillus counts were determined after 48 hours incubation.

## Statistical Methods

The difference in Total CFU, S. mutans, and lactobacilli between the two groups were analyzed by two way analysis of variance for repeated measures. Pairwise contrasts at each time interval were computed by the method of Schaffe. Due to unequal variances, logs of the microbial counts were used for this analysis. Differences in colony forming units between baseline and 1 year for each subject were analyzed by unpaired t-test. Subjects were also ranked according to levels of S. mutans and the difference between these ranked groups was analyzed non-parametrically by the Wilcoxon two-sample ranks test for unpaired measurements.

## Results

Of the 38 subjects who started in the study, 22 remained after 1 year. Of these, only 13 were identified as being completely compliant with the instruction to rinse twice daily over the study year.

Analysis of Total CFU data showed no difference in this parameter between the NaF and SnF$_2$ groups (Table 9). Both groups, however, showed a significant two-fold reduction over baseline values in Total CFU after 1

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year (Tables 9 & 13).

The alternate assignment of subjects by initial salivary S. mutans levels into the acidulated NaF or $SnF_2$ groups produced two closely matched populations with regard to their mean numbers of S. mutans and to their ranking relative to their S. mutans levels (Tables 10 & 11). This match was maintained even though 40% of the subjects dropped out of the study. The mean recoverable salivary S. mutans levels comprised 1.6% of the total recoverable flora in this population at baseline.

After 1 month the number of S. mutans was significantly reduced from baseline in the group rinsing with $SnF_2$, especially in those subjects identified as completely compliant. At 3 months, the reduction of S. mutans in these subjects showed maximum effect. Three subjects compliant with $SnF_2$ mouthrinsing had no detectable S. mutans at this examination. At 6 months, there was a moderate rise in S. mutans levels, which in the compliant subjects, leveled between from 6 months to 1 year. The subjects considered partially compliant with the $SnF_2$ rinsing regimen showed less dramatic reductions in S. mutans counts until the 1 year examinations. At this point, a large drop in recoverable S. mutans was evident (Table 10).

A significant difference in salivary S. mutans levels between the $SnF_2$ and acidulated NaF subjects was evident at the 1 year exam for all degrees of compliance. The entire $SnF_2$ group at 1 year had dramatically lower S. mutans counts from baseline levels, however, no change from baseline was detected in the NaF group (Tables 10 & 13). Of the 12 subjects in the $SnF_2$ group whose recoverable S. mutans levels were greater than $2.0 \times 10^5$/ml saliva at baseline, 10 subjects were noted to have less than 200,000 S. mutans/ml saliva at 1 year. The acidulated NaF group, showed no alteration in S. mutans from their baseline levels (Table 11).

The lactobacilli recoveries showed no significant differences between

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groups or longitudinally within the treatment categories. The variation in number of recovered lactobacilli was very large, even in the same subject at different sampling times. This lack of consistent recovery of lactobacilli is reflected by the standard deviations which were consistently higher than means at all sampling intervals (Table 12).

## Discussion

Certain study design limitations should be recognized when examining the results and evaluating the appropriateness of the statistical tests. The 22 subjects remaining after 1 year do not represent a normally distributed population so these results should not be compared to the population in general. The population is skewed since only those subjects that had high caries prevalence and elevated salivary $S.$ mutans counts were selected as potential subjects, and only approximately half of the subjects who began the study were examined at 1 year. Instead of random assignment into the two treatment groups, subjects were ranked according to $S.$ mutans levels and then alternately assigned to rinse with acidulated NaF or $SnF_2$. This was done because $S.$ mutans levels, the most important variable, vary greatly among individuals and it was desirable to have this variable balanced at baseline due to the relatively few subjects. Furthermore, since the study has no real control group, the small but significant longitudinal change in Total CFU in both the treatment groups cannot be attributed with certainty to a treatment effect.

The change in $S.$ mutans counts are striking even considering the design limitations. $SnF_2$ appears to have a potent long term effect on salivary $S.$ mutans levels. While the effect is most evident in the patients identified as completely compliant with rinsing twice a day, it was also found in

those subjects who were partially compliant with mouthrinse usage. This dramatic reduction in S. mutans may possibly explain why after 1 year the subjects rinsing with $SnF_2$ had half the caries increment as those rinsing with acidulated NaF.

In vitro studies determining minimum inhibitory and lethal concentrations of various fluorides have clearly shown that S. mutans is more susceptible to $SnF_2$ than other fluoride compounds. The large tin accumulation in bacteria exposed to $SnF_2$ may contribute to the greater antimicrobial activity of $SnF_2$.

The apparent selective supression of S. mutans (reduction of S. mutans relative to Total CFU) by $SnF_2$ is also of interest, since S. mutans is now considered the important microorganism for the initiation of dental caries. Other human and animal studies using NaF, instead of $SnF_2$, have also shown selective reductions in S. mutans. Such selectivity of a non-antibiotic against a specific pathogen is not easily understood. One in vitro study using minimium bactericidal concentrations found that S. sanguis and S. mutans had similar sensitivities to fluoride compounds[28]. Furthermore, other effective antiplaque agents have shown only non-specific antimicrobial properties against oral flora. The substantial supression of S. mutans by $SnF_2$ found in this study and the reported selectivity of fluorides in general against S. mutans needs further study. If $SnF_2$ does have a specific effect against S. mutans, then $SnF_2$ would have major advantages over other agents in the treatment of subjects having high caries activity associated with elevated S. mutans levels. The microbial selectivity and the well documented physicochemical effect of $SnF_2$ on tooth enamel would probably make this compound superior to sodium fluoride or antiseptic treatment.

The group rinsing with acidulated NaF showed little change in oral

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flora using the methods and the parameters of this study. This finding is in agreement with others. While elevated concentrations of fluoride are bactericidal mouthrinse levels of NaF appear to have little effect on salivary flora.

The lack of effect of both fluoride agents and dental treatment on lactobacilli was not surprising. Neither chlorhexidine nor fluoride has shown potential in supressing this microorganism. Also in the present study, the great variability of lactobacilli counts even in the same individual, limited the meaningfulness of this parameter. Even though salivary lactobacilli recoveries are known to correlate with caries, this organism is no longer considered as important as $S.$ mutans for the initiation of dental caries.

## Conclusion

Twenty-two subjects, who were regarded as potentially caries active, rinsed twice a day with either acidulated NaF or $SnF_2$ mouthrinses, adjusted to 200 ppm $F^-$. At baseline, and after 1, 3, 6, and 12 months each subject's saliva was analyzed for Total CFU, $S.$ mutans, and lactobacilli.

There was a small (2 times) but significant reduction in Total CFU per ml saliva in both groups after a year. No differences were found in lactobacilli counts between the 2 mouthrinse groups or longitudinally within the groups. Of importance was the apparent selective reduction in $S.$ mutans found in those subjects rinsing with $SnF_2$. While there was essentially no change in $S.$ mutans in the NaF group, all subjects in the $SnF_2$ group had large reductions. At the end of 1 year the $SnF_2$ group had less (26 times) fewer $S.$ mutans compared to baseline. The reduction in $S.$

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_mutans_ levels correlates to the reported lower caries scores in the subjects rinsing with $SnF_2$.

The selective antimicrobial actions of $SnF_2$ against _S. mutans_ and the established physicochemical action of $SnF_2$ with tooth enamel may make this agent superior to other fluoride agents in the treatment of subjects having high caries activity associated with elevated _S. mutans_ levels.

STUDY 4: EFFECT OF $SNF_2$ AND ACIDULATED NAF MOUTHRINSE ON CARIES INCIDENCE
IN ADULTS WITH HIGH NUMBERS OF <u>S. MUTANS</u> AND HIGH CARIES PREVALENCE

## INTRODUCTION

It is well documented that topically applied fluorides decrease caries
activity, but there has been no strong evidence demonstrating the relative
effectiveness of fluoride compounds. Currently, fluoride is believed to be
effective, in part, due to its ability to stimulate remineralization. Other
physicochemical and antibacterial mechanisms may have important anticaries
action as well. The principal fluoride solution used today, NaF, decreases
plaque formation when applied at high concentrations, but not at mouthrinse
levels. Stannous fluoride ($SnF_2$), however, has been shown to have
antibacterial properties at mouthrinse concentrations <u>in vitro</u> and <u>in vivo</u>.

Children with a high caries incidence harbor large numbers of
<u>Streptococcus mutans</u> per ml saliva. When the number of <u>S. mutans</u> is
decreased, the caries incidence is reduced. Even though it has been
suggested that topically applied NaF should be more effective in high risk
children, at least one study has shown that NaF has little effect on
subjects with high caries activity indicating that factors such as
microorganisms and diet cannot be nullified by NaF. $SnF_2$ mouthrinse with
its potential antimicrobial activity, however, has not been tested
specifically on high risk subjects. Furthermore, while it has been shown
that children with high DMFS scores and high numbers of <u>S. mutans</u> are at
risk for caries, such studies in adults are largely lacking.

The aims of this study were: 1. to compare the effectiveness of $SnF_2$
and acidulated NaF in adults with high numbers of <u>S. mutans</u> and high caries

prevalence and 2. to determine if the number of S. mutans and the caries prevalence can be used to predict the incidence of caries in adults.

## MATERIALS AND METHODS

### Subjects

The subjects in this study were adults over the age of 18 living in a fluoridated area. They had incipient carious lesions, high numbers of unrestored carious lesions and elevated numbers of S. mutans in their saliva indicating high caries activity. Of the 58 subjects first identified by their caries prevalence in the screening clinic at the University of Connecticut School of Dental Medicine, subsequent microbial screening showed that 38 subjects had greater than $2 \times 10^5$ S. mutans per ml saliva. These subjects were regarded as potentially highly caries active . The 37 subjects who consented to the study were ranked by their number of S. mutans and then alternately assigned to a $SnF_2$ or an acidulated NaF mouthrinse group. During the first year 15 patients withdrew from the study. Of the remaining 22 patients, 9 were considered partially compliant with the mouthrinsing procedures. Partially compliant subjects were those who, by their report, missed more than 4 mouthrinses per month, or who were inconsistent with mouthrinsing. We verified these reports by monthly monitoring of each patient's remaining supply of mouthrinse and by questioning the patients for monthly recall of their fluoride usage.

### Treatment

After baseline data were obtained, subjects were instructed to use 10 ml of their respective mouthrinses twice daily for 1 minute per rinse. The $SnF_2$ rinse (Iradicav[R], Johnson & Johnson Co., East Windsor, NJ) was diluted

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with water (1:4) immediately before use to produce a final fluoride concentration of 200 ppm F⁻ and a pH of 3.4. The acidulated NaF mouthrinse (Phos-Flur Oral Rinse[R], Hoyt Laboratories, Needham, MA) was used full strength at a fluoride concentration of 200 ppm F⁻ and a pH of 4.0. One month after the initiation of mouthrinsing, each subject received 3 dental hygiene appointments at one week intervals. The oral hygiene instruction, cleaning, scaling and root planing were performed by one dental hygienist, blind to the subjects' treatment categories. The subjects were also assigned to a dental resident for restoration of teeth with active carious lesions. They were contacted monthly to reinforce oral hygiene, to monitor their fluoride usage and to resupply them with mouthrinse.

## Clinical Examinations

Caries recording was performed with the aid of a front surface dental mirror, explorer and posterior bite-wing radiographs. Prior to examination the teeth were cleaned and dried. All decayed, filled and missing surfaces in the permanent dentition were recorded excluding third molars. Incipient smooth surface lesions, not included in the DMFS score, were described by their size. All recordings were performed by the same dentist. The diagnostic error was calculated by duplicate recording performed on 8 subjects, 5 to 14 days after baseline examination. The reproducibility score, i.e. the number of surfaces diagnosed as carious or sound at both examinations, was 93%. At the end of the year the subjects were re-examined for new lesions.

## Microbiology

Stimulated saliva from each subject, produced by chewing on a piece of paraffin wax, was collected at the screening and baseline examinations. Each saliva sample was immediately vortexed, diluted in 0.05M phosphate

buffer (pH 7.0) and plated on Mitis-Salivarius agar containing 0.2 units/ml Bacitracin (MSB) by the micromethod of Westergren and Krasse. The agar plates were incubated for 48 hours in a $CO_2$-enchriched environment (candle jar). The mean count of the two samples was considered the patient's number of  S. mutans/ml saliva.  Subsequent saliva samples were taken at 1, 3, 6, and 12 months.

## Statistical Methods

The differences in caries incidence between the two groups were analyzed non-parametrically by the Wilcoxon two-sample ranks test for unpaired measurements.  All tests were performed at the 0.05 level of significance.

## RESULTS

The subjects, who were alternately assigned to either the acidulated NaF or $SnF_2$ groups by their levels of inital  S. mutans/ml saliva, had other baseline characteristics that were well distributed.  The subjects in the acidulated NaF group presented with means of 7.5 white spot lesions, 15 unrestored carious lesions and a total mean DMF(S) of 63.2.  The group asssigned to rinse with $SnF_2$ had means of 7.8 white spot lesions, 12.7 unrestored carious lesions and a total mean DMF(S) of 76.4 (Tables 14 & 15). Note that partially-compliant and compliant subjects were considered one group in the statistical analyses.

The population of subjects was generally unreliable.  Of the 38 subjects who started the study, 22 were in the study after 1 year and, of these, 13 were identified as being completely compliant with the instructions to rinse twice every day over the study year.

During the year, all subjects who rinsed with acidulated NaF developed new carious lesions.  The mean numbers of new lesions were 4.3

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and 4.5, respectively for the compliant and partially-compliant acidulated NaF rinsing groups. Of the 44 new lesions after 1 year in this group, 10 (23%) were recurrent lesions (Table 15). The caries activity after 1 year in the $SnF_2$ group was significantly lower. The mean numbers of new lesions for the $SnF_2$ group were 2.3 and 2.8 respectively for the compliant and partially-compliant groups. Two subjects who rinsed regularly with $SnF_2$ developed no new lesions, while 1 subject in both the compliant and partially-compliant group developed 5 new lesions. Recurrent decay contributed 28% of the total new lesions in this group (Table 15).

## DISCUSSION

Caries prevalence was used as the primary criterion to select adults with high caries risk, since it has been demonstrated that caries incidence correlates to caries prevalence. In this study, however, the numbers of unrestored and incipient carious lesions were considered more important in identifying high risk patients because Klock and Krasse have shown that these parameters are better correlated to caries incidence than missing and filled surfaces. Based on the clinical data, potential subjects were screened for high numbers of salivary S. mutans since, at least in children, high caries prevalence in combination with high numbers of S. mutans correlates better to caries activity than caries prevalence alone. According to the limits set by Zickert et al. subjects with more than 200,000 S. mutans /ml saliva were considered at high risk and accepted for the study.

Because all subjects received fluoride, it is not possible to show the true caries incidence for this type of population. It is reasonable to assume that our selection process for high risk patients was valid since

both the incidence and the caries prevalence in the two test groups in this study were considerably higher than Lu et al. reported for adults in a nonfluoridated area of the U.S. (DMFS=38; DMFS=0.69/year). It should be noted, however, that Axelsson and Lindhe reported caries prevalence and caries incidence in a Swedish population similar to those in the present study.

The results showed that rinsing with $SnF_2$ was more effective than acidulated NaF in highly caries active patients. A possible explanation for this might be the better antimicrobial effect of $SnF_2$, demonstrated by the lowered S. mutans levels in the $SnF_2$ group. This is in agreement with a study by Zickert et al. who showed that another antibacterial agent, Chlorhexidine gluconate, reduced both S. mutans and caries incidence in children with high caries activity. Thus, highly caries active patients screened by means of elevated S. mutans may be effectively treated by agents directed against the pathological microorganisms. It should be noted that even though the $SnF_2$ rinsing reduced the caries incidence more than acidulated NaF, the remaining caries activity was still high. Similar finding have been reported by Seppä et al.

Aside from the number of S. mutans, high caries activity can be the result of low enamel resistance, high sucrose intake and/or low salivary flow. With factors other than elevated pathogenic microorganisms, $SnF_2$ may not be better than other fluoride compounds at reducing caries activity. The effect of these other factors may explain why highly caries active patients given the same treatments may be affected differently.

We cannot tell if the acidulated NaF treatment and/or oral hygiene appointments had any effect on caries incidence in this population due to the lack of a control population. Both a positive effect and a poor effect

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for NaF treatment have been reported in highly caries active children. It is obvious, though, that a finding of 4.4 new lesions after 1 year in the acidulated NaF group is extremely high, and consequently the daily acidulated NaF regimen appears to have had only a limited effect.

A surprising finding in this study was that those patients who were identified as strictly adhering to the regimen (rinsing twice a day) did not seem to develop fewer lesions than those who were known to use the mouthrinse less frequently. This finding is in contrast to other studies showing that frequent fluoride exposures are inversely related to development of new lesions. Possibly either our criterion for strict compliance (missing less than 4 rinsings out of 60 per month), or a more than optimium fluoride regimen, could contribute to the lack of difference between the compliant and partially compliant subjects.

The drop-out figure (45%) was very high. No study similar to this one has been reported and perhaps rinsing twice a day is excessive behavioral change for patients who have high caries experience.

In conclusion, this study found that highly caries active patients could be identified by means of caries prevalence and salivary S. mutans levels. All patients continued to be caries active after one year despite the use of two daily fluoride mouthrinses; however, the subjects rinsing with $SnF_2$ developed approximately half the number of new carious lesions to those subjects rinsing with acidulated NaF.

STUDY 5:  EFFECT OF $SNF_2$ AND ACIDULATED NAF MOUTHRINSES
ON PLAQUE AND GINGIVITIS IN ADULTS WITH HIGH CARIES PREVALENCE

## INTRODUCTION

Efforts to prevent or treat periodontal diseases are aimed at the control of plaque, either through mechanical or chemical means.  Based on numerous short and long term studies, the antiseptic chlorhexidine is now the most used adjunct in treating these diseases.  Short term studies also have demonstrated SnF2 to be effective in plaque reduction when used as a mouthrinse twice daily.  Only one study has examined the use of SnF2 on a long term basis. In this study, school children rinsing once a day for 4 months with SnF2 had lower plaque scores than those rinsing with NaF, but no difference was found in gingivitis scores between the two groups.

As part of a comprehensive study on adults with rampant caries adjunctively rinsing with either SnF2 or NaF, we examined their plaque and gingivitis levels longitudinally.  This report discribes the differences between the two groups in the periodontally related parameters after 1 year.

MATERIAL AND METHODS

The subjects for this study were selected by their high caries prevalence, and by their high numbers of salivary S. mutans. Of the initial 37 subjects, 22 remained in the study after 1 year. The subjects were further catagorized as compliant or partially compliant. Partial compliance was defined as those who, by their own report, missed more than 4 mouthrinses per month, or who were inconsistant with their mouthrinsing. These reportrs were verified by monitoring the patients remainig supply of mouthrinse and by monthly questioning of the patients regarding their fluoride usage.

Following baseline examination, subjects were instructed to use 10 ml of their mouthrince twice daily for one minute per rinse. The SnF2 rinse (Iradicav, Johnson and Johnson Co., East Windsor, N.J.) was diluted with water (1 part SnF2: 4 parts water) immediately before use to produced a final fluoride concentration of 200 ppm and a pH of 3.4. The acidulated NaF mouthrinse (Phos-Flur Oral Rinse, Hoyt Laboratories, Needham, Mass.) was used at full strength at a concentration of 200 ppm fluoride and a pH of 4.0. One month after the initiation of mouthrinsing, each subject had 3 dental hygience visits at one week intervals. Oral hygience instruction, prophylaxis, and scaling and root planning were performed by one dentral hygenist, blind to the patients treatment catagory. The subjects also received complete restorative treatment by dental residents. In order to reinforce oral hygience, monitor fluoride usage and resupply the subjects with fluoride mouthrinse, they were contacted monthly.

The baseline and 1, 3, 6, and 1 year subsequent examinations included

gingival index and plaque index which were recorded by one examiner, blind as to the subjects groupings. The GI and PI1 data was reduced to frequency of scores and means for each subject. The frequency scores were then converted to "% of sites with plaque" (ie. PI1 score of 1,2,or3), and to "% of sites with bleeding" (ie. GI score of 2 or 3). Individual percentages were then averaged for each group. The scores for each subject were also converted to mean GI and PI1 and then analyzed as parametric data using analysis of variance for repeated measures. This test enabled longitudinal as well as cross sectional evaluation of each group.

RESULTS

The subjects in this investigation represented essentially a poorly compliant population. Sixteen subjects dropped out in the first year; and of the remaining 22, only 13 were considered compliant in their use of the mouthrinse.

After one year, both the $SnF_2$ and the NaF "Total" (compliant and non-compliant subjects) groups demonstrated a significant decrease in plaque (mean differences of 66% and 62%, respectively. However, no significant difference was found in plaque scores betweeen the $SnF_2$ and NaF groups (Table 16).

Gingival inflammation was significantly reduced in the Total $SnF_2$ group, both longitudially from baseline (23%), and cross sectionally compared to the Total NaF group (17%). Statistical differences in the GI reduction between groups is seen in the Total and Compliant groupings, but not in the Non-compliant subjects when analyzed separately (Table 17).

A further analysis was performed to correlate individuals mean plaque score to their mean gingival score. Plaque scores were positively correlated to gingival scores in those subjects rinsing with NaF ($r = .83$); however, in the $SnF_2$ group the subjects plaque scores had a significant inverse relationship to the gingival scores ($r = -.57$).

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DISCUSSION

The reduction in plaque scores from baseline to 1 year in both the SnF2 and NaF rinsing groups was expected sine all subjects had thorough instruction and reinforcement in oral hygiene. This significant improvement in the subjects oral hygiene and the eventual low plaque scores probably hinders the possibility of observing differences in plaque scores between groups. Furthermore, several studies have described a increased in non-bacterial pellicle on tooth surfaces of subjects rinsing with SnF2. Since pellicle is not readily distinguishable from plaque with clinical indices, this tooth deposit would also confound plaque scores. Evidence that subjects in the SnF2 group had non-bacterial deposits on their teeth is suggested by the negative correlation between the plaque scores and the gingival scores in the SnF2 group.

This study did find that SnF2 was an adjunct in decreasing gingival inflamation. The lower frequency of bleeding sites and the corresponding lower mean GI scores in the SnF2 group compared to the NaF group demonstrates that rinsing with SnF2 favorably affected gingival health. Since we have also noted decreased caries, and greatly reduced S. mutans in these same subjects rinsing with SnF2 compared to NaF, SnF2 may have advantages at least in subjects with dental disease. This noted effect of SnF2 on gingivitis levels differs from the only other long term examining this parameter. Perhaps our population of adults with existing gingivitis, or the fact that our subjects rinsed twice a day, seven days a week could account for the differences.

Theoritically SnF2 has the potential of affecting gingival health.

Studies in vivo have shown that SnF2 irrigated into periodontal pockets dramatically reduces the number of presumptive periodontopathic microorganisms and gingival bleeding, and in vitro studies have shown that SnF2 is more effective than other fluoride compounds in inhibiting growth and viablity of B. melogenicus, and Actinomyces species.

From several short term studies showing antiplaque effects of SnF2 and the present study showing a small but significant affects on gingivitis, frequent rinsing with SnF2 may have greater effect than other fluoride rinses in treatment of subjects with high caries activity and periodontal disease. However, as we observed in this study it may be difficult for the average patient to follow a regime of twice a day rinsing over an extended period. Perhaps other delivery systems or regimens that don't require as much patient cooperation are necessary as a large scale public health measure.

STUDY 6: SAFTEY AND ANTIBACTERIAL PROPERTIES OF

CONTROLLED RELEASE SNF$_2$

INTRODUCTION

Sustained release delivery of drugs has several advantages: (1) it enable lower dosage of drugs because the agent is released near the intended site of action, (2) it overcomes problems of side effects because of the lower therapeutic levels; (3) it reduces need for patient envolvement; and (4) it eliminates the need for frequent drug administration.

In dentistry sustained release systems have been explored for delivery of steroids, anti-fungal drugs; antibacterials ; and fluorides for the control of dental caries and remineralization. To date the largest a clinical study has been performed with a trilaminate methacrylate sodium fluoride-releasing device cemented to the buccal surfaces of teeth of 11 subjects. The intraoral device was found to elevate the levels of fluoride in plaque, saliva and urine, but had no effect on plaque or gingival parameters.

Fluoride ions may act as a therapeutic agent by altering bacterial metabolism as well a reacting physicochemically with enamel to reduce enamel solubility or remineralize initial caries. Stannous fluoride has been shown to have a greater effect on bacterial metabolism than other fluoride compounds. Pilot studies with stannous fluoride incorporated in

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polycarboxylate cement and used as a temporary intercoronal restoration have been performed to test its potential as an antiplaque device. These studies, performed $\underline{in\ vitro}$ and in one subject, demonstrated that the $SnF_2^-$ polycarboxylate cement had sufficient compressive strength, released of fluoride at therapeutic levels, had acceptable clinical properties, and caused a visual change in plaque formation .

Based on these favorable initial experiments, the present clinical study was performed to further evaluate the saftey and efficacy of the $SnF_2^-$polycarboxylate intercoronal restoration in two human clinical trials.

## MATERIALS AND METHODS

### Safety and Fluoride Release

Eight subjects were used to test: I. the integrety of the $SnF_2$ polycarboxylate cement as an intracoronal restoration; 2. the potential side effects; 3. the oral release of fluoride from the cement, and 4. the charge of fluoride levels in the urine. Subjects consisted of dental students and dental assistants who consented to the study and had a defective restoration in a molar tooth requiring at least a two surface restoration. The trial period, when the slow release restoration was in place, lasted 34 days.

A week prior to the trial and during the trial period, all subjects were given fluoride free toothpaste and instructed to use only this toothpaste until the experiment was over.

The $SnF_2$-polycarboxylate cement was prepared by combining pulverized $SnF_2$ with polycarboxylate powder (Durelon, Premier) in a ratio of I:I (W/W). The fluoride crystals were pulverized to a fine powder by triturating the compound in a dental amalgamator (Wiggle-bug, LPGO, Cresent Dental) for I minute at maximum velocity.

The test tooth in each subject was prepared conventionally for an intracoronal restoration. Orthodontic bands were then fitted around the test tooth. (The bands were used to prevent tooth drift in case the temporary restoration was lost prematurely.) The $SnF_2$-polycarboxylate powder was mixed with the polycarboxylate liquid according to manufacturers instructions and used to cement the orthodontic band around the tooth as well as restoring the tooth. Approximately 300mg $SnF_2$ (72mg $F^-$) was used in each restoration. The teeth were kept dry throughout all procedures with the aid of rubber dam isolation. After the cement had hardened, the

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rubber dam was removed and occlusion of the restoration was adjusted. Besides giving the subjects instructions about giving saliva and urine samples, the subjects maintained normal oral hygiene and activity. The temporary restorations were photographed, approximately 1:1, at the time of placement and again at the end of the trial period. At the end of the trial, the slow release restorations were replaced with either amalgam or gold restorations.

Twice prior to the trial period and twice weekly during the trial period, salivary and urinary samples were collected between 8 and 10 a.m. Salivary samples consisted of whole saliva, stimulated by having subjects chew on parafin. Saliva and urine samples were diluted 1:1 with ionic strength buffer (TISAB with CDTA). The fluoride ion concentrations of the samples were then determined using a combination fluoride electrode (Orion, model 96-09) connected to a digital readout electrometer. Millivolt readings of the samples were compared to those of NaF standards.

## Efficacy

Fourteen subjects were alternately assigned into 2 groups so that half had a controlled release $SnF_2$ restoration placed in a molar and half had a placebo restoration (IRM). As in the previous trial the subjects were dental students or assistants who required two or more surface restoration in a permanent molar.

A two week initial preparation period, in which all subjects were scaled, polished and given detailed instructions in effective plaque control, preceded the experimental period. On day one of the experimental period a temporary restoration of either 50% $SnF_2$ in polycarboxylate cement or IRM was placed within a cavity preparation of a tooth of the appropriate subject. The procedure was identical to the previous trial except that orthodontic bands were not used this time around the restoration. Subjects

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were then instructed to abstain from all forms of active oral hygiene for the next 14 days. Following the 14 days trial period, permanent restorations were placed in the test teeth, oral hygiene was reinstituted, and each subject received a profesional tooth cleaning. Subjects continued to be followed for 2 weeks after the experimental period (Figure 6).

Microbiologic baseline samples were taken twice before the trial (one week prio and immediately before the temporary restoration was placed). Microbiologic samples from each subject were again taken on day 7 and 14 of the trial and twice during the post-trial period. The microbiologic procedures consisted of enumerating total colony forming units, S. sanguis, and S. mutans from salivary samples. Stimulated saliva from each subject was collected as previously described in the morning for each designated period. One ml of saliva from each subject was diluted with 3 ml of reduced transport fluid without ethylenediamine tetraacetate, sonicated for 10 sec. at output setting 4 (Bronson, B15, with microtip), and then serially diluted. Samples were plated onto enriched trypticase soy blood agar, MM10 with 20% sucrose and HLR with 20% sucrose and 0.2 units bacitracin. Special sectors containing 20 to 50 colonies were quantitated for total colony forming into units, S. sanguis and S. mutans, respectively. S. sanguis and S. mutans were identified by morphologic criteria. Questionable colonies of S. mutans were subjected to biochemical analysis. The mean of 2 samples for each time interval was used to distrbute each subject into ranges with regard to total colony forming units, S. sanguis and S. mutans.

Clinical measurements of gingivitis-G.I. and plaque-PI1 were taken on each subject immediately prior to placement of the temporary restoration and measurements were repeated on days 7, 14, and 28. The mean frequency

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of scores "0" and "2" for each subject were used to determine the group mean scores of "0" and "2" at each scoring period.

## RESULTS

### Safety and Fluoride Release

Of the 8 subjects who had the $SnF_2$-polycarboxylats temporary restorations placed in a molar tooth, 7 completed the 34 day trial. One subject was discontinued after 5 days because the proximal portion of the restoration broke. All the subjects on the first day experienced pain and gingival irritation where the orthodontic band and $SnF_2$- polycarboxylate cement that was used to cement the orthodontic band touched the gingiva. Because of the irritation, the orthodontic band and cement that held the band was removed from all subjects after the second day.

The baseline salivary fluoride levels for all subjects was less than 0.5 ppmF⁻. The salivary fluoride levels increased to a mean of 1.5 ppm on the second day after the restoration was in place. The salivary fluoride levels sharply declined on day 6 to a mean of 0.5 ppm and then gradually decreased during the rest of the trial. Detectable levels of fluoride above baseline were still found at 4 weeks. Over the course of the 34 day trial the mean salivary fluoride level was 0.3 ppmF (Figure 7).

Baseline urinary fluoride levels were approximately 0.7 ppmF. On the second day of the trial the mean urinary fluorida level of the subjects was 2.2 ppm. Urinary fluoride levels were only slightly above baseline after day 6 (Figure 8).

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Except for a delay in the setting time (approximately 15 minutes), the $SnF_2$-polycarboxylate combination was similar to that of unaltered polycarboxylate cement. Excluding the one broken restoration, the integrity of the filling material was excellent during the trial period. There was no noticeable wear of the restoration on the margins or on the proximal contact. All restorations, however, had a change in color from white to dark grey (Figure 9).

## Efficacy

Subjects receiving the $SnF_2$-polycarboxylate cement and whose cavity design extended subgingivally again experienced pain and gingival irritation lasting 2-3 days. Gingival sloughing of the sulcular epithelium appeared to have occurred in 2 subjects who had preparations extending several millimeters subgingivally.

Categorization of subjects by the number of total colony forming units/ml saliva revealed a shift to increased number of bacteria in those subjects receiving the control cement during the experimental period. No increase in total CFU was observed in those subjects having the $SnF_2$ restoration (Table 18). Subjects distributed by S. mutans recoveries at the different intervals revealed that during the experimental period there was a shift upward in S. mutans in those subjects having the placebo while there was a shift downward in those subjects having the $SnF_2$ restoration (Table 19). As with total CFU, the S. sanguis counts increased during the experimental period and decreased in the post-experimental period (Table 20). However, no difference between the $SnF_2$-polycarboxylate group and the placebo group could be noted at any time interval.

Plaque scores of all the subjects were excellent at baseline as shown by the high number of "0's" and the low number of "2's". After 7 days of no oral hygiene in the experimental period, both groups had a similar high

number of "2" scores. At 14 days, the frequency of score "2" was 16% less in the $SnF_2$ group. At the end of the 14 day post-trial period, the large number of "0" scores indicated reinstitution of excellent oral hygiene practices, but the placebo group showed a 10% higher number of "0" scores (Table 21).

The gingival health of the subjects was also excellent as shown by the high frequency of "0" GI scores in both groups. Little change in gingival health was noted until the end of the trial period (2 weeks without oral hygiene). No difference, however, was detected in gingivitis levels at this time. The post-trial GI scores showed that all subjects' gingival health returned to baseline levels (Table 22).

## DISCUSSION

The present clinical trials were designed to examine the safety and efficacy of a controlled release delivery system of $SnF_2$ in a small number of human subjects. Although the study designs do not permit statistical tests of significance, certain trends are apparent.

The compatibility of large quantities of $SnF_2$ in polycarboxylate cement, as shown in a previous pilot study is apparent. The $SnF_2$ restorations showed no signs of wear or loss of integrity in both the trials. The release of $SnF_2$ from polycarboxylate cement as measured by the salivary fluoride levels over a 34 day period, was similar to release patterns of other drugs from controlled release devices . There was an initial large release of fluoride in the first days followed by a an linear decline in salivary fluoride levels over month period. The mean fluorice levels over the month period of 0.3 ppm F may have been less than optimal. This release rate, however, may be realistic relative to having only one

restoration in the mouth of each subject. The finding of only minor increases in urinary fluoride levels further substantiates the systemic safety of this release system.

The side effect of gingival irritation and consequent patient discomfort where the restoration touched gingival tissues can not be ignored. Both $SnF_2$ and acidulated NaF have been reported to cause irritation, of crevicular epithelium especially in the presence of gingival inflamation. In this delivery system, where gingival tissues are irritated due to operative procedures, inflamation of the gingiva surrounding the tooth receiving the temporary restoration can not be avoided. However, in the future, avoiding soft tissue contact of the $SnF_2$-polycarboxylate restoration may be possible by placing this restoration only in situations where it does not touch the gingiva, (i.e. Class I restorations), or by protecting the gingiva with an orthodontic band cemented around the tooth with a non-fluoride containing cement prior to placement of the controlled release restoration.

Some effect on both the quantity and proportion of microorganisms was noted in those subjects who had a $SnF_2$-polycarboxylate restorations in place. While there was an increase of recovery of total colony forming units from salivary in the placebo group during the experiemental period, probably due to suspension of oral hygiene in this period, a decrease in total bacteria was noted in the $SnF_2$ group. This decrease in salivary microorganisms may be selective since S. sanguis recoveries showed no difference between group but S. mutans recoveries appeared less in those subjects having the $SnF_2$ restoration. This selectivity of $SnF_2$ against S. mutans has been previously observed.

The effect of the $SnF_2$ delivery system against plaque and gingivitis was not immpressive. Only at one data point, the frequency PLI score 2 at

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day 14, showed a reduction of 16% for those subjects who had the $SnF_2$ controlled release devices in place. We had little expectation of finding differences between groups with regard to these parameters. The large pellicle deposits produced by $SnF_2$ interfere with traditional plaque scoring methods. Other measurement systems which take this problem into account should be used when visualizing plaque in a study using $SnF_2$. Gingivitis scores were not different between the $SnF_2$ group and the placebo group. However, the study period of only 2 weeks does not permit enough time for gingivitis to develop in experimental gingivitis model. Longer term studies using $SnF_2$ at higher concentrations in animals and humans have shown reduced gingivites due to $SnF_2$.

These present studies must be compared to the results found with a recently reported study with controlled release NaF. In that study 2 membrane controlled release NaF. In that study, two membrane controlled devices containing 42 mg each of NaF (total of 38 mg $F^-$/subject) were cemented to first molar of 11 subjects. Side effects in that study were that 2 devices fell off and several subjects reported that the device caused irritation to soft tissues. The membrane controlled devices did increase fluoride levels in saliva and plaque, but no changes could be found in clinical plaque, gingivitis or microbial parameters.

Obviously, devices for the controlled release of fluoride need more development and clinical trials before they can be accepted as a therapeutic approach on a population basis. Further clinical trials that have reduced tissue irritation, increase the amount and duration fo fluoride release and greater bacterial effect need to be achieved. Perhaps use of $SnF_2$ in these release systems, because of its apparent selectivity against S. mutans and employment of an intercoronal delivery system because

the quantity of fluoride compounds can be increased in subjects requiring restorations of multiple teeth, should be further explored.

| Agent | % | Cation (ppm) | Anion (ppm) | pH's |
|---|---|---|---|---|
| $H_2O$ | --- | --- | --- | 2.5, 6.0* |
| NaF | 0.055 | 303 | 250 | 2.5, 3.0, 5.5*, 6.0, 7.0 |
| $SnF_2$ | 0.103 | 783 | 250 | 2.0, 2.5, 3.0, 3.5*, 4.0, 5.0, 6.0, 7.0 |
| $SnCl_2$ | 0.124 | 783 | 463** | 2.5*, 7.0 |
| $SnF_4$ | 0.064 | 390 | 250 | 2.3 |
| $ZnF_4$ | 0.068 | 428 | 250 | 5.2* |
| $CuF_2$ | 0.067 | 418 | 250 | 3.0, 6.0 |
| $PbF_2$ | 0.065 | 545 | 100*** | 3.0, 6.0 |

*unadjusted pH
**$SnCl_2$ prepared to be cationicly equal to that of $SnF_2$.
***$PbF_2$ not soluble at 250 ppm F.

Table 1: List of agents tested against S. mutans. Percentage of compounds prepared to give same anion concentration.

| Agent | Agent pH | Acid Production ($\Delta$ pH) | Plaque Score[a] | Plaque Weight (mg) | Metal/mg Plaque ($\mu$g) |
|---|---|---|---|---|---|
| NaF | 5.5 | 2.7 | 3 | 9.8 $\pm$ 0.6 | N.D. |
| ZnF$_2$ | 5.2 | 2.8 | 3 | 10.0 $\pm$ 0.5 | 0.05 $\pm$ 0.01 |
| SnF$_4$ | 2.3 | 2.6 | 3 | 10.9 $\pm$ 0.2 | 8.9 $\pm$ 2.0 |
| SnF$_2$ | 3.5 | 0.4 | <1 | 1.3 $\pm$ 0.4 | 39.1 $\pm$ 1.4 |

$\alpha$ Scored by McCabe method

N.D. = Non-detected

N=3; $\bar{x} \pm$ S.D.

Table 2: Effect that twice daily exposure of listed fluoride compounds (250 ppm F⁻) had on several growth parameters and metal uptake of S. mutans NCTC 10449.

| Agent | Agent pH (adjusted) | Acid Production ($\Delta$ pH) | Plaque Score[a] | Plaque Weight (mg) | Metal/mg Plaque ($\mu$g) |
|---|---|---|---|---|---|
| $H_2O$ | 2.5 | 2.8 | 4 | 11.0 ± 0.2 | N.D. |
|  | 7.0 | 2.7 | 4 | 10.7 ± 0.5 | N.D. |
| NaF | 2.5 | 2.5 | 4 | 12.5 ± 0.5 | N.D. |
|  | 7.0 | 2.7 | 4 | 11.2 ± 0.9 | N.D. |
| $SnCl_2$ | 2.5 | 2.7 | 4 | 12.3 ± 0.6 | 1.4 ± 0.6 |
|  | 7.0 | 2.7 | 4 | 11.1 ± 0.7 | 0.3 ± 0.1 |
| $SnF_2$ | 2.5 | 1.7 | 2 | 7.2 ± 2.0 | 13.6 ± 4.1 |
|  | 7.0 | 2.6 | 4 | 13.0 ± 0.5 | 0.5 ± 0.1 |

a Scored by McCabe method.

N.D. = Non-detected

N=3; $\bar{x}$ · S.D.

Table 3: Effect that twice daily exposure of fluoride compounds (250 ppm $F^-$) or controls adjusted to low or neutral pH, had on S. mutans NCTC 10449 growth and metal uptake.

| Agent | Agent pH (adjusted) | Acid Production ($\Delta$ pH) | Plaque Score$\alpha$ | Plaque Weight (mg) | Metal/mg Plaque ($\mu$g) |
|---|---|---|---|---|---|
| | 2.0 | 0.2 | 1 | 1.8 ± 0.1 | $\lambda$ |
| | 3.0 | 0.2 | 1 | 2.4 ± 0.5 | 42.9 ± 7.1 |
| $SnF_2$ | 4.0 | 0.5 | 1 | 2.6 ± 0.5 | 36.9 ± 3.6 |
| | 5.0 | 1.0 | 3 | 5.7 ± 0.4 | 20.1 ± 0.5 |
| | 6.0 | 1.6 | 4 | 5.9 ± 0.8 | 3.6 ± 0.7 |

$\alpha$ Scored by McCabe method.

$\lambda$ Laboratory accident.

$N = 3$; $\bar{x} \cdot$ S.D.

Table 4: Effect that twice daily exposure of $SnF_2$ (250 ppm $F^-$) at various pH's had on S. mutans NCTC 10449 growth and metal uptake.

| Agent | Agent pH (adjusted) | Acid Production ($\Delta$ pH) | Plaque Score[a] | Plaque Weight (mg) | Metal/mg Plaque ($\mu$g) |
|---|---|---|---|---|---|
| NaF | 3.0 | 1.9 | 3 | 5.7 ± 0.2 | N.D. |
| | 6.0 | 2.3 | 3 | 4.6 ± 1.0 | N.D. |
| PbF$_2$ | 3.0 | 1.9 | 3 | 6.7 ± 0.3 | 3.3 ± 0.5 |
| | 6.0 | 2.0 | 3 | 5.5 ± 0.1 | 2.4 ± 0.7 |

a Scored by McCabe method.

N.D. = Non-detected.

N = 3; $\bar{x}$ ± S.D.

Table 5: Effect that twice daily exposure of NaF (250 ppm F⁻) or PbF$_2$ (100 ppm F⁻) had on S. mutans NCTC 10449 growth and metal uptake.

| Agent | Agent pH (adjusted) | Acid Production ($\Delta$ pH) | Plaque Score[a] | Plaque Weight (mg) | Metal/mg Plaque ($\mu$g) |
|---|---|---|---|---|---|
| NaF | 3.0 | 2.39 | 5 | 13.3 ± 0.9 | N.D. |
|  | 6.0 | 2.33 | 5 | 12.4 ± 1.9 | N.D. |
| $CuF_2$ | 3.0 | 2.42 | 5 | 12.7 ± 1.6 | Trace |
|  | 6.0 | 2.48 | 5 | 13.0 ± 0.8 | Trace |

[a] Scored by McCabe method.

N.D. = Non-detected.

$N = 3$; $\bar{x}$ ± S.D.

Table 6: Effect that twice daily exposure of NaF or $CuF_4$ (250 ppm F⁻) had on S. mutans NCTC 10449 growth and metal uptake.

| Agent | Agent pH | Acid Production (Δ pH) | Plaque Score[a] | Plaque Weight (mg) | Metal/mg Plaque (μg) |
|---|---|---|---|---|---|
| $H_2O$ | 6.0 | 2.2 | ↺ | 8.3 ± 1.0 | N.D. |
| $SnF_2$ | 3.5 | 1.3 | 3 | 12.2 ± 0.7 | 9.7 ± 2.5 |

a Scored by McCabe method.

N.D. = Non-detected

N=4; $\bar{x}$ ± S.D.

Table 7: Effect that twice daily exposure of $SnF_2$ (250 ppm $F^-$) had on preformed S. mutans NCTC 10449 plaque.

| Tx | n | CARIES SCORE | | | | MICROBIAL RECOVERY |
| | | ENAMEL | Red. | DENTINAL | Red. | X10$^6$ |
|---|---|---|---|---|---|---|
| H$_2$O | 13 | 6.54 ± 1.80 † | — | 3.23 ± 2.45 | — | 37.6 ± 36.0 |
| NaF | 14 | 3.85 ± 1.51* | 41% | 0.57 ± 0.94* | 82% | 19.9 ± 21.4 |
| SnF$_2$ | 13 | 3.69 ± 2.10* | 44% | 0.46 ± 0.78* | 86% | 27.6 ± 32.5 |

† $\bar{x}$ ± S.D.
* Significantly different from deionized H$_2$O group, p ≤ .05

Table 8

Caries Scores and S. Mutans Recovery in Hamsters Drinking
Deionized H$_2$O, NaF, or SnF$_2$ (5 ppm F$^-$) for 64 Days.

| | Agent | n | Baseline* | 1 Month | 3 Months | 6 Months | 12 Months |
|---|---|---|---|---|---|---|---|
| **Complete Compliance** | NaF | 6 | 28.8 ± 29.5 | 10.6 ± 3.1 | 20.5 ± 23.9 | 11.6 ± 7.5 | 11.3 ± 5.7 |
| | SnF$_2$ | 7 | 20.3 ± 12.8 | 17.4 ± 18.5 | 5.8 ± 3.6 | 15.8 ± 17.1 | 6.6 ± 5.7 |
| Differences† | | | | -- | -- | -- | -- |
| **Partial Compliance** | NaF | 4 | 21.7 ± 10.5 | 18.6 ± 9.4 | 22.2 ± 14.2 | 29.1 ± 35.4 | 14.0 ± 10.3 |
| | SnF$_2$ | 5 | 19.4 ± 9.5 | 16.5 ± 9.2 | 18.5 ± 27.0 | 10.2 ± 7.1 | 15.9 ± 23.8 |
| Differences† | | | | -- | -- | -- | -- |
| **Total Group** | NaF | 10 | 26.0 ± 23.1 | 13.8 ± 7.2 | 21.2 ± 19.6 | 18.6 ± 23.0 | 12.5 ± 7.6 |
| | SnF$_2$ | 12 | 20.0 ± 11.1 | 17.0 ± 14.7 | 12.0 ± 18.7 | 13.5 ± 13.6 | 10.5 ± 15.7 |
| Differences† | | | | -- | -- | -- | -- |

* $\bar{x} \pm$ S.D.
† Non-significant

TABLE 9

Number of Total CFU ($\times 10^7$) in different groups at
baseline and after 1, 3, 6, and 12 months.

60

| | Agent | n | Baseline* | 1 Month | 3 Months | 6 Months | 12 Months |
|---|---|---|---|---|---|---|---|
| **Complete Compliance** | | | | | | | |
| | NaF | 6 | 4.2 ± 4.0 | 5.7 ± 6.2 | 6.6 ± 9.5 | 8.2 ± 9.3 | 3.9 ± 4.9 |
| | $SnF_2$ | 7 | 3.1 ± 1.5 | 0.1 ± 0.1 | 0.02 ± 0.03 | 0.4 ± 0.5 | 0.3 ± 0.3 |
| Differences[†] | | | | 57x | 366x | --- | --- |
| **Partial Compliance** | | | | | | | |
| | NaF | 4 | 2.3 ± 1.6 | 3.8 ± 2.8 | 2.9 ± 1.0 | 4.3 ± 2.4 | 4.5 ± 4.1 |
| | $SnF_2$ | 5 | 5.7 ± 3.3 | 1.2 ± 2.2 | 1.4 ± 1.2 | 4.8 ± 4.1 | 0.04 ± 0.05 |
| Differences[†] | | | | --- | --- | --- | 111x |
| **Total Group** | | | | | | | |
| | NaF | 10 | 3.4 ± 3.3 | 5.0 ± 2.2 | 5.1 ± 7.4 | 6.6 ± 7.3 | 4.1 ± 4.4 |
| | $SnF_2$ | 12 | 4.2 ± 2.7 | 0.6 ± 1.4 | 0.7 ± 1.1 | 2.0 ± 3.2 | 0.2 ± 0.3 |
| Differences[†] | | | | 9x | 8x | --- | 26x |

* $\bar{x}$ ± S.D.

† Differences shown are significant, $p < .1$ by analysis of variance

TABLE 10

Number of S. mutans ($x10^6$) in different groups at baseline and after 1, 3, 6, and 12 months.

61

| S. *mutans*/ml saliva | Baseline | | 1 Year* | |
|---|---|---|---|---|
| | NaF | SnF$_2$ | NaF | SnF$_2$ |
| > 5 million | 3 | 4 | 4 | --- |
| 3-5 million | 2 | 2 | 1 | --- |
| 1-3 million | 3 | 5 | 2 | --- |
| 200,000-1 million | 2 | 1 | 2 | 2 |
| < 200,000 | --- | --- | 1 | 10 |

*Differs significantly, p< .05 by 2-sample ranks test

TABLE 11

Ranking of all subjects within treatment groups by S. <u>mutans</u> levels at baseline and after 1 year.

| Agent | n | Baseline* | 1 Month | 3 Months | 6 Months | 12 Months |
|---|---|---|---|---|---|---|
| **Complete Compliance** | | | | | | |
| NaF | 6 | 1.0 ± 1.7 | 0.7 ± 0.6 | 3.0 ± 5.4 | 1.2 ± 2.8 | 1.6 ± 3.7 |
| SnF$_2$ | 7 | 1.3 ± 1.5 | 1.8 ± 3.7 | 0.5 ± 0.8 | 0.7 ± 1.1 | 1.8 ± 3.2 |
| Differences† | | | - - - | - - - | - - - | - - - |
| **Partial Compliance** | | | | | | |
| NaF | 4 | 0.5 ± 0.5 | 2.1 ± 3.9 | 1.3 ± 1.2 | 0.7 ± 0.8 | 1.5 ± 2.8 |
| SnF$_2$ | 5 | 0.5 ± 0.6 | 0.6 ± 0.9 | 1.4 ± 2.3 | 2.7 ± 3.4 | 0.8 ± 1.6 |
| Differences† | | | - - - | - - - | - - - | - - - |
| **Total Group** | | | | | | |
| NaF | 10 | 0.8 ± 1.3 | 1.3 ± 2.4 | 2.3 ± 4.2 | 1.0 ± 2.1 | 1.5 ± 3.2 |
| SnF$_2$ | 12 | 1.0 ± 1.3 | 1.3 ± 3.0 | 0.9 ± 1.6 | 1.5 ± 2.4 | 1.4 ± 2.6 |
| Differences† | | | - - - | - - - | - - - | - - - |

\* $\bar{x}$ ± S.D.

† Non-significant

TABLE 12

Number of lactobacilli ($\times 10^6$) in different groups at baseline and after 1, 3, 6, and 12 months.

63

|  |  |  | Reductions* | |
| Microorganism | Agent | n | Times | Percent |
|---|---|---|---|---|
| Total C F U | NaF | 10 | 2.1x | 52% |
|  | SnF$_2$ | 12 | 1.9x | 48% |
| S. *mutans* | NaF | 10 | — | — |
|  | SnF$_2$ | 12 | 21x | 95% |
| Lactobacilli | NaF | 10 | — | — |
|  | SnF$_2$ | 12 | — | — |

*Reductions shown are significant, $p < .05$, by t-test

TABLE 13

Reductions in all subjects' salivary micro-organisms at 1 year.

64

| Subject | DMF | Initial White Spots | D | M | F | (%) | Primary Caries | Recurrent Caries | Root Caries | Σ | Attack Rate |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 8 | 13 | 24 | 31 | = 68 | 1 | -- | -- | 1 | 1.0 |
| | | 6 | 7 | 4 | 0 | = 11 | 7 | -- | -- | 7 | 5.6 |
| 595 | | | 19 | 25 | 6 | = 50 | 4 | -- | -- | 4 | 4.1 |
| 602 | | 7 | 11 | 0 | 56 | = 67 | 2 | 3 | -- | 5 | 3.9 |
| 503 | | 10 | 21 | 29 | 40 | = 90 | 2 | -- | -- | 2 | 2.0 |
| 504 | | 8 | 12 | 0 | 85 | = 97 | 3 | 4 | -- | 7 | 5.5 |
| | x̄ = 27.6 | x̄ = 7.5 | x̄ = 15.0 | x̄ = 15.1 | x̄ = 63.2 | | Subtotal x̄ = 4.3 | | | | x̄ = 3.7 |
| 203 | 19 | 15 | 24 | 15 | 9 | = 48 | 7 | -- | -- | 7 | 6.2 |
| 316 | 6 | 3 | 11 | 50 | 4 | = 65 | -- | 1 | -- | 1 | 1.3 |
| 602 | 53 | 7 | 24 | 4 | 62 | = 90 | 1 | 2 | 1 | 4 | 3.2 |
| 603 | 18 | 7 | 8 | 0 | 38 | = 46 | 6 | -- | -- | 6 | 4.7 |
| | | | | | | | Subtotal x̄ = 4.5 | | | | x̄ = 3.9 |
| | | | | | | | Total x̄ = 4.4 | | | | x̄ = 3.8 |

New Lesions After 1 Year

Baseline

Table 14

Caries prevalence at baseline and caries incidence after 1 year in the acidulated NaF group.

**Baseline**

| Subject | Age | Initial White Spots | D | M | F (S) | Primary Caries | Recurrent Caries | Root Caries | Σ | Attac Rate |
|---|---|---|---|---|---|---|---|---|---|---|
| 102 | | 11 | 5 | 0 | 28 = 33 | 2 | -- | -- | 2 | 2.0 |
| 1-- | | 5 | 4 | 35 | 34 = 73 | 2 | -- | 1 | 3 | 3.2 |
| 10? | | 2 | 24 | 22 | 29 = 75 | 2 | -- | -- | 2 | 1.9 |
| 114 | | 12 | 13 | 15 | 32 = 60 | -- | -- | -- | 0 | 0 |
| 118 | | 14 | 7 | 0 | 69 = 76 | -- | 5 | -- | 5 | 3.9 |
| 701 | | 12 | 36 | 39 | 10 = 85 | -- | -- | -- | 0 | 0 |
| 70? | | 12 | 25 | 14 | 51 = 90 | 4 | -- | -- | 4 | 3.5 |
| | x̄ = ?3.? | x̄ = 7.8 | x̄ = 12.7 | x̄ = 21.8 | x̄ = 76.4 | | Subtotal | | x̄ = 2.3 | x̄ = 2.1 |

**Partial Compliance:**

| Subject | Age | Initial White Spots | D | M | F (S) | Primary Caries | Recurrent Caries | Root Caries | Σ | Attac Rate |
|---|---|---|---|---|---|---|---|---|---|---|
| 103 | 3? | 6 | 6 | 15 | 59 = 80 | 5 | -- | -- | 5 | 4.4 |
| 303 | 2? | 5 | 2 | 15 | 86 = 103 | 1 | -- | -- | 2 | 1.8 |
| 307 | ?? | 2 | 16 | 35 | 40 = 91 | 1 | 1 | -- | 2 | 2.2 |
| 40? | ?? | 12 | 5 | 42 | 31 = 78 | -- | 1 | -- | 1 | 1.2 |
| 404 | ? | 1 | 9 | 30 | 34 = 73 | 3 | 1 | -- | 4 | 4.1 |
| | | | | | x̄ = 76.4 | | Subtotal | | x̄ = 2.8 | x̄ = 2.7 |
| | | | | | | | Total | | x̄ = 2.5* | x̄ = 2.4* |

Statistically different from NaF group.

### Table 15

Caries prevalence at baseline and caries incidence after 1 year in the SnF$_2$ group.

|  | Baseline | 1 Month | 3 Months | 6 Months | 12 Months |
|---|---|---|---|---|---|
| **NaF** | | | | | |
| Compliant (6) | 0.7 ± .29* | 0.5 ± .34 | 0.4 ± .16 | 0.4 ± .34 | 0.4 ± .22 |
| Non-Compliant (4) | 1.0 ± .78 | 0.8 ± .82 | 0.3 ± .27 | 0.4 ± .24 | 0.3 ± .08 |
| Total (10) | 0.8 ± .53 | 0.6 ± .55 | 0.4 ± .21 | 0.4 ± .29 | 0.3 ± .17 |
| **SnF$_2$** | | | | | |
| Compliant (7) | 0.5 ± .32 | 0.3 ± .36 | 0.3 ± .29 | 0.3 ± .23 | 0.2 ± .28 |
| Non-Compliant (5) | 0.8 ± .61 | 0.7 ± .47 | 0.2 ± .13 | 0.5 ± .18 | 0.3 ± .19 |
| Total (12) | 0.6 ± .47 | 0.5 ± .43 | 0.3 ± .23 | 0.4 ± .22 | 0.2 ± .24 |

*$\bar{x}$ ± S.D.

Table 16

Mean Plaque Score (PlI) for Compliant, Non-Compliant, and Total Subjects at Each Examination.

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|  | Baseline | 1 Month | 3 Months | 6 Months | 12 Months |
|---|---|---|---|---|---|
| NaF |  |  |  |  |  |
| Compliant (6) | 1.3 ± .24* | 1.1 ± .13 | 1.2 ± .19 | 1.3 ± .19 | 1.2 ± .14 |
| Non-Compliant (4) | 1.3 ± .35 | 1.3 ± .36 | 1.1 ± .03 | 1.1 ± .03 | 1.2 ± .09 |
| Total (10) | 1.3 ± .27 | 1.2 ± .24 | 1.1 ± .16 | 1.2 ± .17 | 1.2 ± .12 |
| SnF$_2$ |  |  |  |  |  |
| Compliant (7) | 1.3 ± .21 | 1.2 ± .14 | 1.0 ± .09 | 1.1 ± .07 | 1.0 ± .12+ |
| Non-Compliant (5) | 1.4 ± .26 | 1.3 ± .26 | 1.0 ± .13 | 1.2 ± .14 | 1.1 ± .07 |
| Total (12) | 1.3 ± .22 | 1.3 ± .20 | 1.0 ± .10 | 1.2 ± .12 | 1.0 ± .11+ |

*$\bar{x}$ ± S.D.

+Significant differences, $p < .05$ between treatment groups over time.

Table 17

Mean Gingival Scores (G.I.) for Compliant, Non-Compliant, and Total Subjects at Each Examination.

| | Baseline Period | | Experimental Period (no oral hygiene) | | Post-Experimental Period | |
|---|---|---|---|---|---|---|
| | Control | SnF$_2$ | Control | SnF$_2$ | Control | SnF$_2$ |
| 10⁹-10¹⁰ | 0 | 0 | 2 | 1 | 0 | 0 |
| 10⁸-10⁹ | 5 | 5 | 5 | 3 | 3 | 3 |
| 10⁷-10⁸ | 2 | 2 | 0 | 3 | 4 | 4 |

Table 18 Number f subjects ordered by total salivary colony forming units at baseline, experimental and post-experimental periods. The mean of 2 samples was used to categorize each subject at each period.

| | Baseline Period | | Experimental Period (no oral hygiene) | | Post-Experimental Period | |
|---|---|---|---|---|---|---|
| | Control | SnF$_2$ | Control | SnF$_2$ | Control | SnF$_2$ |
| >10$^5$ | 2 | 3 | 3 | 0 | 2 | 2 |
| 10$^4$-10$^5$ | 2 | 2 | 3 | 4 | 3 | 3 |
| 10$^3$-10$^4$ | 1 | 0 | 0 | 1 | 1 | 0 |
| <10$^3$ | 2 | 2 | 1 | 2 | 1 | 2 |

Table 19 Number of subjects ordered by salivary S. mutans counts at baseline, experiments, and post-experimental periods. The mean of 2 samples was used to categorize each subject at each period.

| | Baseline Period | | Experimental Period (no oral hygiene) | | Post-Experimental Period | |
|---|---|---|---|---|---|---|
| | Control | SnF$_2$ | Control | SnF$_2$ | Control | SnF$_2$ |
| $10^8$-$10^9$ | 0 | 0 | 0 | 0 | 0 | 1 |
| $10^7$-$10^8$ | 2 | 3 | 7 | 6 | 4 | 3 |
| $10^6$-$10^7$ | 5 | 4 | 0 | 1 | 3 | 3 |

Table 20 Number of subjects ordered by salivary S. sanguis counts at baseline, experimental and post- experimental periods. The mean of 2 samples was used to categorize each subject at each period

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| | % Frequency Score 0 | | % Frequency Score 2 | |
|---|---|---|---|---|
| | Placebo | SnF$_2$ | Placebo | SnF$_2$ |
| Baseline | 89 | 82 | 3 | 0 |
| Day 7 | 3 | 4 | 72 | 75 |
| Day 14 | 1 | 2 | 79 | 66 |
| Post-Trial | 91 | 81 | 1 | 1 |

Table 21   Mean frequency per subject of   scores 0 and 2 in subjects having slow release SnF2 restorations (N=7) or a placebo restoration (N=7) of a molar tooth for 14 days.
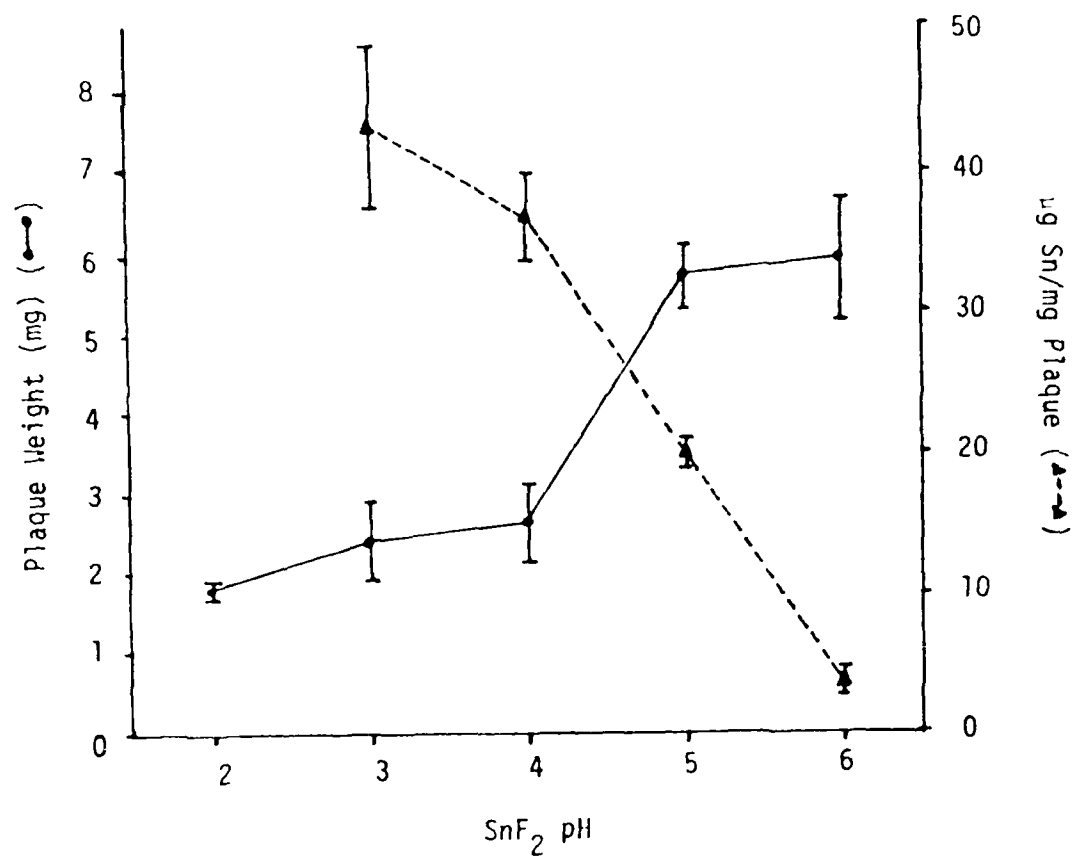
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| | % Frequency Score 0 | | % Frequency Score 2 | |
|---|---|---|---|---|
| | Placebo | SnF2 | Placebo | SnF2 |
| Baseline | 82 | 88 | 0 | 2 |
| Day 7 | 80 | 82 | 0 | 2 |
| Day 14 | 46 | 44 | 6 | 9 |
| Post-Trial | 81 | 87 | 0 | 1 |

Table 22   Mean frequency per subject of GI scores 0 and 2 in subjects having slow release SnF2 restoration (N=7) or a placebo restoration (N=7) of a molar tooth for 14 days.

Figure 1: Increasing the pH of $SnF_2$ decreases its effectiveness as shown by the greater plaque accumulation on wires and the reduced tin per mg plaque.
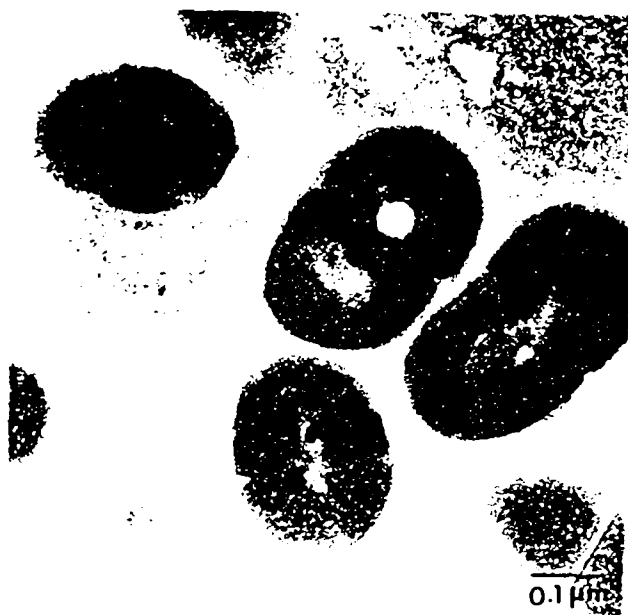
Figure 2:  Electron micrograph of S. mutans exposed to NaF (250 ppm F⁻).
Unstained, X80,000
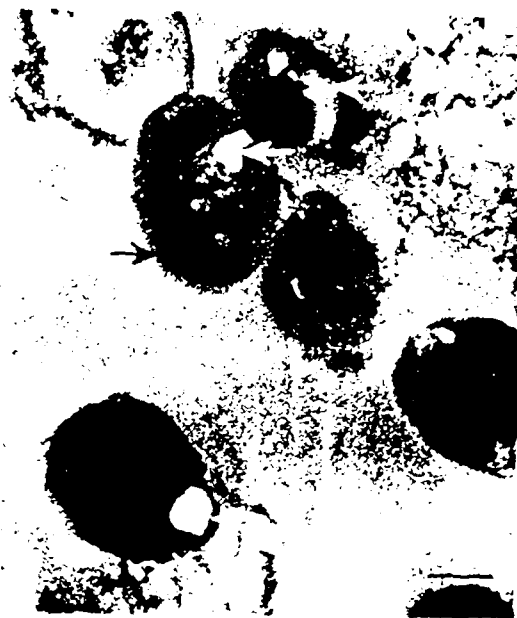
Figure 3: Electron micrograph of S. mutans exposed to SnF$_2$ (250 ppm F$^-$). Note frequent intracellular electron dense granules (black arrows) and electron lucent holes (white arrows). Unstained, X80,000
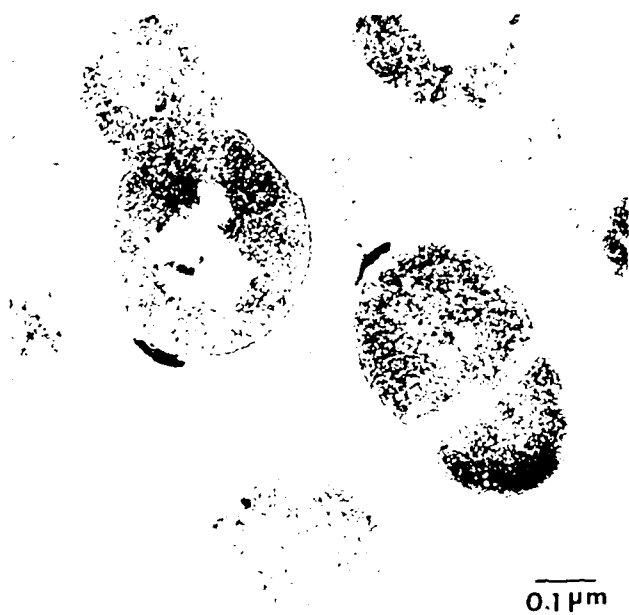
0.1 μm

Figure 4:  Electron micrograph of S. mutans exposed to PbF$_2$ (100 ppm F$^-$).
Note electron dense granules outside the bacteria.
Unstained, X80,000
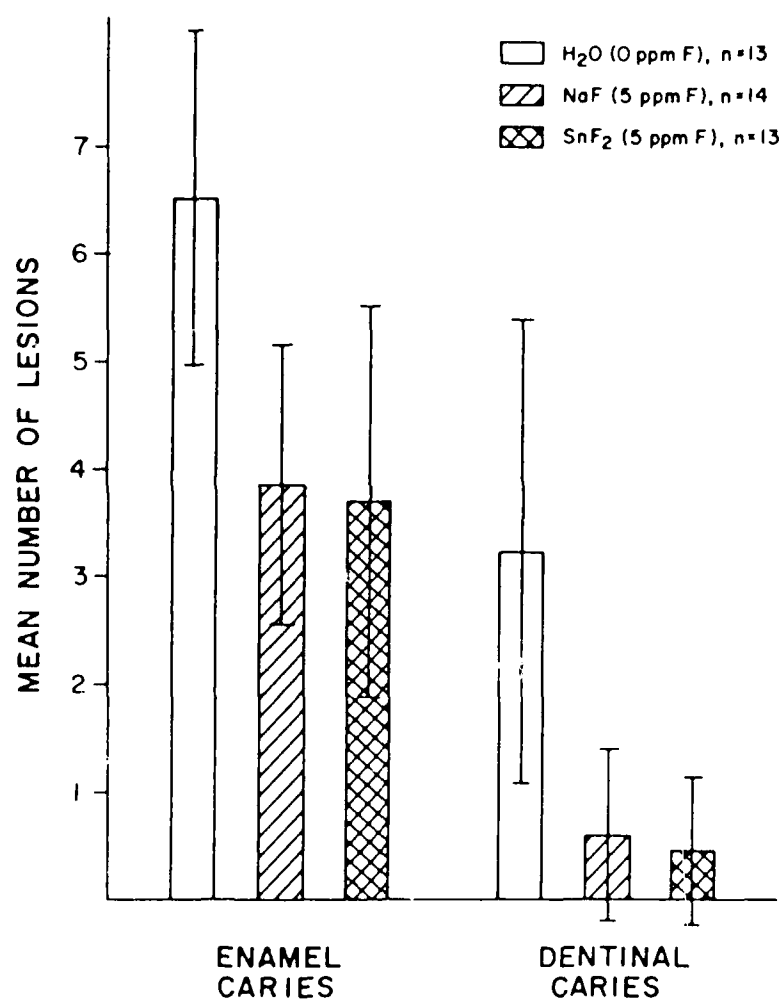
Figure 5:  Means and standard deviations of enamel and dentinal carious lesions per hamster drinking either deionized $H_2O$, NaF, or $SnF_2$ (5 ppm F-). Experimental caries was produced by oral inoculation of streptomycin-resistant S. mutans NCTC 10449 and by NIH diet 2000. Exposure to fluoride started when animals were approximately 38 days old to provide essentially a topical effect.
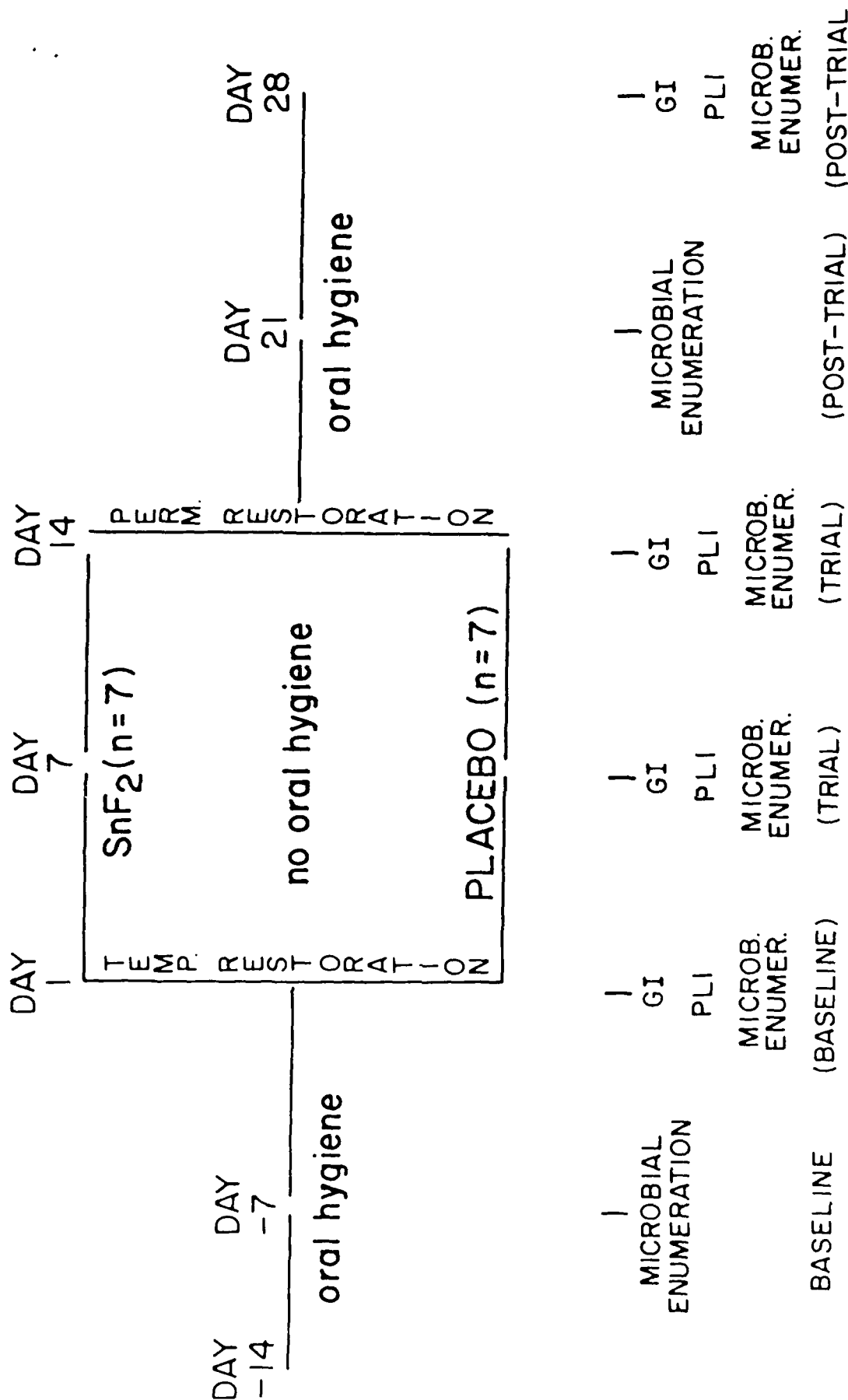
Figure 6: Experimental design for efficacy trial. After an initial preparation period of 14 days, subjects suspended oral hygiene and received either a SnF2 polycarboxylate restoration or a placebo restoration in 1 molar tooth. Following 14 more days, oral hygiene was reinstituted and permanent restorations were placed. Data collection was taken twice during the initial preparation, trial, and post-trial periods.
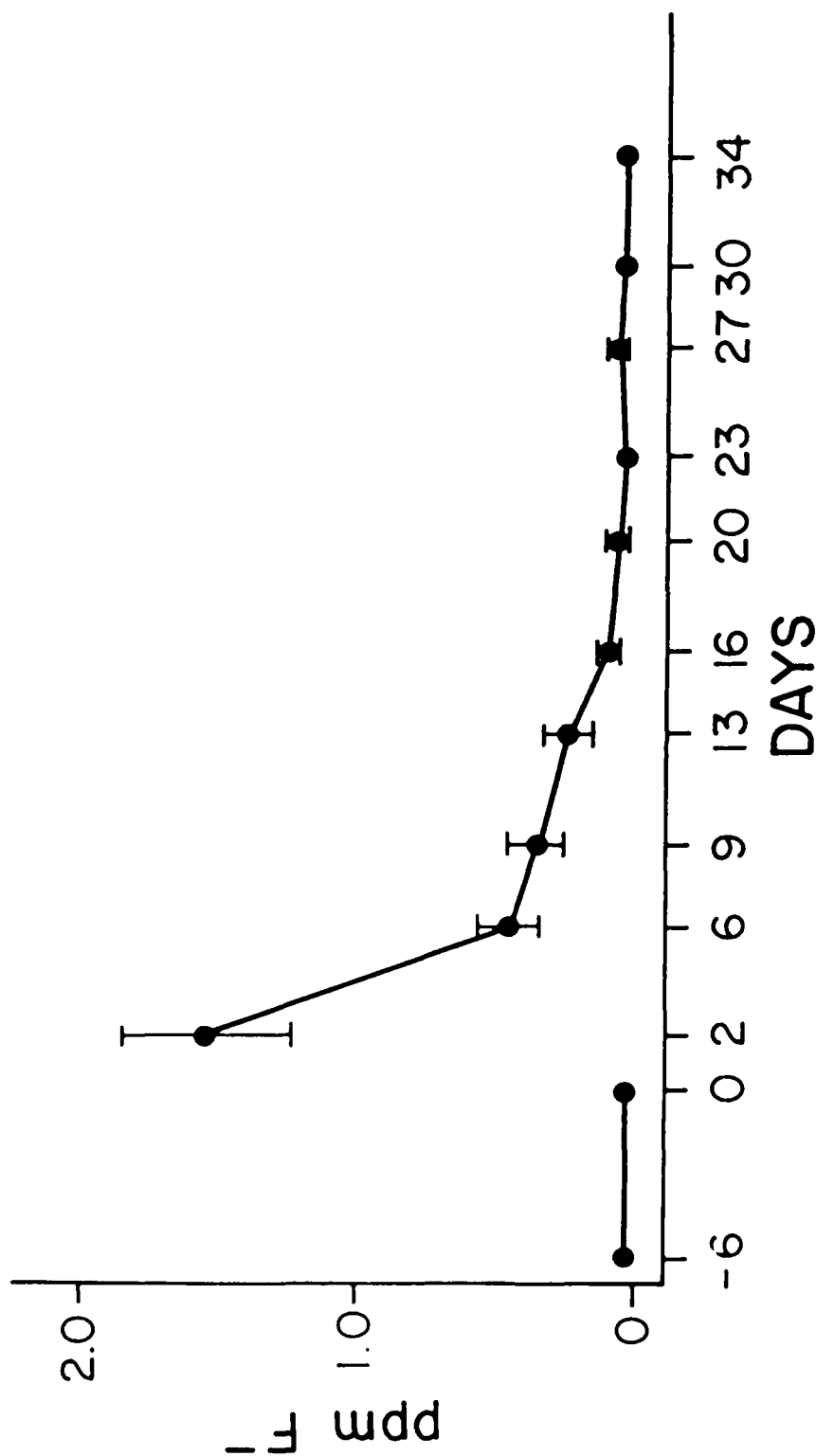
Figure 7: Salivary fluoride levels (mean and S.D.) from 7 subjects in the trial for safety. SnF2-polycarboxylate cement was placed in a molar tooth on day 0 and removed on day 34. Trial was preceded by 2 baseline levels.
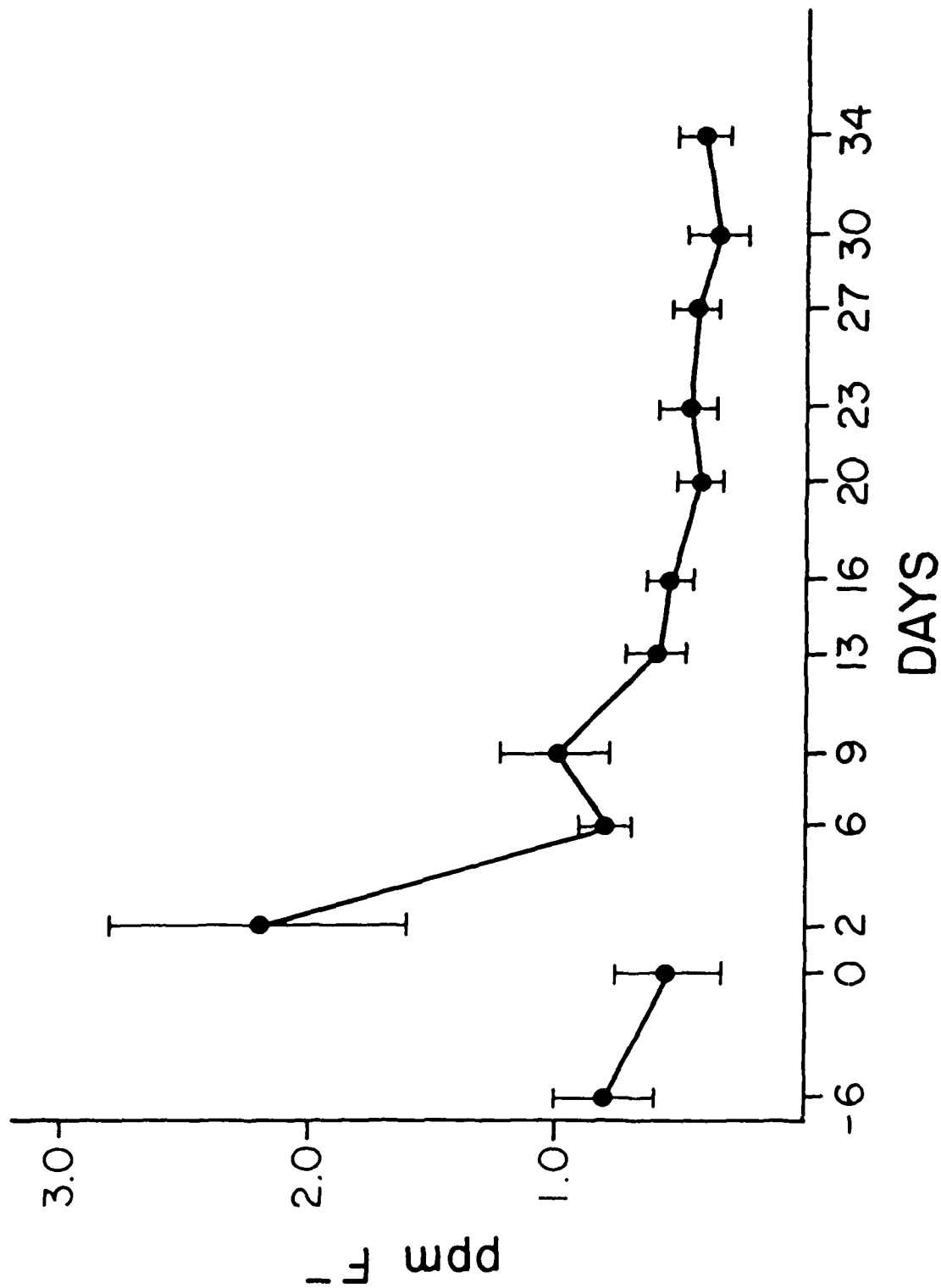
Figure 8: Urinary fluoride levels (mean and S.D.) from 7 subjects in the trial for safety. SnF2-polycarboxylate cement was placed in a molar tooth on day 0 and removed on day 34. Trial was preceded by 2 baseline levels.

Figure 9: Condition of the $SnF_2$-polycarboxylate restoration after 34 days in a "MOD" cavity preparation in a first permanent molar.

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